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TYLOSES: THEIR OCCURRENCE AND PRACTICAL SIGNIFICANCE IN SOME AMERICAN WOODS

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GENERAL DESCRIPTION OF TYLOSES

The large open pores or vessels conspicuous in hardwoods frequently become closed by growths called tyloses.[1] These growths render the wood practically impermeable to air and liquids. On the split surfaces of a wood such as white oak or pignut hickory the tyloses appear in the vessel channels as glistening cellular growths resembling masses of soap bubbles. (Pl. LII, fig. 1.) These masses are protrusions from the living parenchyma cells of the wood itself into adjacent vessel or tracheid cavities. They enter at the thin places or pits in the wall of the wood elements (see Pl. LII, figs. 2 and 3), and expand to a greater or less degree. In the softwoods (Pl. LVI, fig. 1) tyloses are relatively small, but in the hardwoods they frequently form bladderlike sacs of considerable size (Pl. LII, figs. 2 and 3, and Pl. LIII, figs. 1, 2, and 3), often developing simultaneously in many of the parenchyma cells surrounding the tubelike vessel cavities. (Pl. LII, fig. 3.) Under such circumstances, if growth is vigorous, the tylosal sacs, after pushing into the vessel cavity, grow together, completely filling it. In this way the ability of the vessel to conduct air or liquid is effectually checked. (Pl. LIII, figs. 1 and 2.) Sometimes, however, the tylosal growths do not entirely fill the vessel, and only a clogging action results.

The purpose of this study was to determine the occurrence of tyloses in the most important commercial species of native woods and their significance in relation to the adaptability of these woods to certain practical uses.

Observations were made not only of the presence or absence of tyloses in a species, but also of the extent and degree of development and the regions (sapwood or heartwood) where the growths are found.

[1] These growths received, in 1845, the name "Thylle" (tyloses) from a German botanist who signed as "Ungenannte," or "unknown," the paper discussing them. This writer is, however, believed by Boehm and Winkler to have been Frl. Hermine von Reichenbach. The name "Thylle" is derived from the Greek word θύλος, meaning a purse or sack. The occurrence of tyloses was, however, noted as early as 1675 by Malphigi, in the drawing of a cross section of chestnut wood. They are also given the descriptive name "Füllzellen," or filling cells, by the Germans.

Only a brief discussion is given of the causes leading to the formation of tyloses or of their function in the living plant, since studies for this purpose have already been made by other investigators.

MORPHOLOGICAL RELATIONS OF TYLOSES IN WOODY TISSUE

ORIGIN AND DEVELOPMENT

A tylose can not be considered as a distinct cell, for as a rule a cell is defined as a body consisting of cell substance, cell wall, and cell nucleus. With very rare exceptions (Molisch)[1] a tylose, as found in woody tissue, is not completely surrounded by a wall and has no nucleus. It is only a portion or prolongation of a wood or medullary-ray parenchyma cell. (Pl. LII, figs. 2 and 3; Pl. LVII, fig. 2.) Frequently more than one tylose is formed from one parenchyma cell, but only one active nucleus—that of the parenchyma cell—is present, though this may be found in one of the tyloses. (Pl. LII, fig. 3.) A parenchyma cell which has given rise to two tyloses is shown in Plate LII, figure 2.

The growing or arching out of tyloses has been found to follow a reduction in internal pressure or cessation in sap conduction in the large vessels. When this occurs, the living parenchyma cells, which possess a considerable growth potential, expand and press into the adjacent empty vessel cavities. In pitted vessels this expansion is localized in the thin unlignified membranes of the one-sided bordered pits which are present on the dividing walls between vessels or tracheids, and parenchyma (De Bary; Green; Haberlandt; Hanausek; Molisch; Rees; Russow; Sachs; Strasburger; and Winckler). These membranes contain plasma and therefore possess the power of growth. The internal pressure of the turgid parenchyma cells, when exerted against these relatively thin spots or pits, causes the pit membranes to stretch and grow by intussusception[2] (Green; Molisch). The protrusions increase gradually in size and finally develop into the characteristic bladder-shaped sacs known as tyloses. An open passage through the space previously occupied by the unstretched closing membrane of the pit is formed in this way between the tylose and the parenchyma cell. (Pl. LII, fig. 2.) The contents of the tylose are therefore the same as those of the parenchyma cell.

NORMAL AND ABNORMAL TYLOSE FORMATION

It has been shown beyond doubt that the wounding of trees through cuts or bruises or at the points where branches are broken off tends to stimulate tylose formation, and throughout the study this mode of tylose formation has been constantly borne in mind. Generally, however, tyloses are not due to wounding. They are a characteristic feature of the normal uninjured wood of many families of trees. Nevertheless,

[1] Bibliographic citations in parentheses refer to "Literature cited," pp. 468–469.

[2] "Intussusception" means in botany, according to Nageli, the growth of cell walls by the irregular interposition of new solid particles between those already in existence.

the wood produced by felling the tree may have an important bearing on the presence of tyloses in the outer rings of a log, where the parenchyma cells are still living and capable of growth. It is possible to find in these rings young or old, or large and small, tyloses together in the same vessel. (Pl. LIV, R_3.) Although exceptions have been noted, the idea that a considerable number of the outer rings are entirely free from tyloses has, however, been very generally accepted (Strasburger).[1] The data obtained from the present study show that there is a very considerable formation of tyloses in the outer rings of the sapwood. The question then arose as to whether these sapwood tyloses were of normal origin or whether they were due to some wound stimulus, such as the felling of the tree. It was finally concluded that they were normally formed tyloses, because their development throughout the vessels was very uniform instead of being sporadic or irregular, as in the case of tyloses associated with wounds (Pl. LIV, R_1 and R_2), and because an examination of branches from living trees of Rhus, the sumach, Catalpa, and Robinia, the black locust, made immediately after cutting, confirmed the other observations of the relatively early formation of tyloses in many species. In material which was not received for examination until several weeks after it was cut, thin, irregularly distributed tyloses were often found in the outer vessels, though the latter must have been functioning in sap condition at the time the tree was felled.

It is noteworthy also that in this study tyloses were found to reach the most remarkable development in ring-porous woods, such as oak, hickory, black locust, or osage orange. (Pl. LIII, figs. 1 and 3, and Pl. LVI, fig. 2.) In woods where tyloses are few and scattered there is considerable variation from specimen to specimen in the actual number of tyloses present. This tendency is clearly shown in the woods of the diffuse porous group. (Table II.) It is also noticeable that in the two or three rings surrounding the pith in a diffuse porous wood tyloses are often much more abundant than elsewhere in either the heartwood or sapwood.

EFFECT OF THE DISTRIBUTION OF PARENCHYMA TISSUE

Since tylose formation depends upon the presence of parenchyma cells either in the form of wood parenchyma or medullary rays in close proximity to vessels or tracheids, the variation in position, abundance, and vitality of these cells affords at least a partial explanation of the irregular development of tyloses in different species of wood. Parenchyma tissue is considerably developed in the following families and their respective genera.[2] This study has shown that in these families are a large number of native woods exhibiting tyloses.

[1] Tyloses are . . . instrumental in closing the water courses of the heartwood. . . . These are intrusive growths from living cells which penetrate the cavities of the adjoining tracheal elements during the transition of sapwood into heartwood.

[2] Solereder, Hans. Systematic Anatomy of the Dicotyledons . . . v. 2, p. 1143. Oxford, 1908. Certain other woods with abundant parenchyma frequently produce gummy substances rather than tyloses.

Family.	Genera.
Cupuliferæ or Fagaceæ	Castanea, Fagus, Quercus.
Juglandaceæ	Hicoria, Juglans.
Papilionaceæ	Robinia.
Magnoliaceæ	Liriodendron, Magnolia.
Moraceæ	Morus, Toxylon.

The arrangement of wood parenchyma cells in the annual ring has been divided [1] into three different types, as follows:

1. Terminal parenchyma, which is situated at the periphery of the annual growth ring, on the outer face of the summer wood.

2. Metatracheal or diffuse parenchyma, which is scattered among the other elements in the ring, usually forming tangential bands.

3. Paratracheal or vasicentric parenchyma, or parenchyma cells, aggregated around the vessels.

TABLE I.—*Native woods grouped according to the degree of tylose development and the most marked distribution of wood parenchyma in ring.*[2]

ABUNDANT TYLOSES.[3]

Species.	Type of parenchyma.	Species.	Type of parenchyma.
Catalpa speciosa	Paratracheal.	Hicoria orata	Paratracheal.
Chilopsis linearis	Do.	Juglans cinerea	Do.
Morus rubra	Do.	nigra	Do.
Rhus hirta	Do.	Quercus alba	Do.
Robinia pseudacacia	Do.	garryana	Do.
Toxylon pomiferum	Do.	lyrata	Do.
Hicoria alba	Do.	lobata	Do.
aquatica	Do.	macrocarpa	Do.
glabra	Do.	michauxii	Do.
laciniosa	Do.	minor	Do.
minima	Do.	platanoides	Do.
myristicaeformis	Do.	densiflora	Do.
odorata	Do.	marilandica	Do.

MANY TYLOSES.

Castanea dentata	Metatracheal.	Fraxinus lanceolata	Paratracheal.
Celtis occidentalis	Paratracheal.	profunda	Do.
Eucalyptus globulus	Do.	quadrangulata	Do.
Fagus atropunicea	Metatracheal.	Sassafras sassafras	Do.
Fraxinus americana	Paratracheal.		

SCATTERED TYLOSES.

Aesculus vetandra	Scanty paratracheal.	Platanus occidentalis	Metatracheal.
Liquidambar styraciflua	Metatracheal.	Populus grandidentata	Terminal.
Liriodendron tulipifera	Terminal.	tremuloides	Do.
Magnolia acuminata	Do.	trichocarpa	Do.
fraseri	Do.	Ulmus alata	Paratracheal.
glauca	Do.	americana	Do.
		pubescens	Do.

[1] Jeffrey, E. C. A Natural Classification of Woods.
Holden, Ruth. Some features in the anatomy of the Sapindales. In Bot. Gaz., v. 53, no. 1, p. 50–58, pl. 2–3. 1912.

[2] The data here given concerning the distribution of parenchyma were obtained from: (1) Solereder, Hans, op. cit.; (2) Jeffrey, E. C., op. cit.; and (3) from original observations made during the study.

[3] By "abundant" is meant a very large number. "Many" is used to signify a considerable number but less than "abundant."

These three types of arrangement and the degree of their development bear a definite relation to the development of tyloses, since they indicate whether the parenchyma cells are near enough to the vessel cavities to send their prolongations into them. In addition to the wood parenchyma, the position and number of the medullary rays adjacent to the vessels must be taken into account. A grouping of the species of wood with the twofold object of indicating the distribution of tyloses and the arrangement of the wood parenchyma clearly brings out some of the reasons why tyloses are so much more abundant in certain woods than in others. Wherever the paratracheal or vasicentric type of parenchyma is well developed, the tendency for marked tylose formation, or else for gum production, is very noticeable. From Table I it is further evident that when tyloses are strongly developed either paratracheal or abundant metatracheal parenchyma is always found.

SHAPE, THICKNESS OF WALL, AND CONTENTS OF TYLOSES

The shape of the tylosal projections varies widely. They are sometimes spherical, or again they appear as elongated vesicles. (Pl. LII, fig. 3; and Pl. LIII, figs. 1, 2, and 3.) Often when the walls are very thin they appear much collapsed and wrinkled as, for instance, in ash or the wound tyloses in cow oak. (Pl. LIV, *R*1.) The extent to which the tylose wall increases in thickness varies also. The wall may be an extremely thin delicate membrane as found in ash or osage orange (Pl. LV, fig. 2) or it may be of medium thickness as in oak. (Pl. LIII, figs. 1 and 2.)

The contents of the tyloses are in general the same as those of the parenchyma cells producing them. Starch is common, and resin, calcium crystals, and gums have also been observed.

When normal parenchyma cells do not give rise to tyloses, the so-called "gums" (Prael)[1] are often produced, as in mesquite, maple, or cherry. This gum usually collects in the vessels (Pl. LIII, fig. 4) and parenchyma cells. In the vessels it sometimes assumes the form of globules or droplets which may easily be mistaken for tyloses. In order to determine whether gum or tyloses are present, a section of the wood may be treated with some gum solvent, such as absolute alcohol or caustic soda. When the wood is dry, the gum droplets are often characteristically cracked and split. Their general appearance is illustrated in Plate LIII, figure 4.

MATERIAL AND METHODS USED IN THE STUDY

The material used for this study of tyloses was a collection of logs of commercial size from native-grown trees. As a basis for the study of tyloses this material was unique, since most of the work of other investigators has been done not on wood from the bole of the tree, but on

[1] "Schutzgummi."

branches, twigs, roots, leaves, vines, herbaceous plants like the squash, or on such of the lower forms as ferns,[1] and did not cover to any extent the American species.

The method of examining the wood was as follows: The ends of the logs which form the collection of commercial American woods (Pl. LIX, fig. 1) of the Forest-Products Laboratory were examined with a hand lens. Blocks cut from these were also studied microscopically. Small strips extending from the bark through the trees to the pith, including the sapwood, the so-called transition region, and the heartwood,[2] were cut from the logs. Microtome sections about 1 inch by one-half inch in area and 5 to 20 micromillimeters in thickness were cut from the three planes, transverse, radial, and tangential, taken from each of these different regions and were studied under the compound microscope. The observations for hardwoods are given in Table II. Stains were often employed to differentiate the tissues, and macerations were made with potassium hydroxide or chromic acid for special studies of the relations between the tylose and the parenchyma cell producing it. Fresh material from seedlings and branches was also examined, in order to determine whether the sapwood tyloses were of normal or abnormal origin.

The Forest-Products Laboratory collection of woods begun in 1910 is not yet complete, and in many cases only one log of a species was available for study. Nevertheless, the majority of the commercially important species are included in the laboratory collection, and in addition to the study of these it was possible to make further observations on authentic material of a number of other important species. Moreover, whenever two or more specimens of the same species were examined, results were

[1] This list of the plant genera where tyloses have been found in wood, roots, leaves, or other portions is given by Küster. It includes Molisch's observations on the Vienna wood collection and other material as well as those of other authors, whose names are given in parentheses after the genera they investigated.

Abies (Raatz).
Achyranthes.
Aesculus (Maüle, Tison).
Alnus (Tison).
Ampelopsis.
Aralia.
Aristolochia (Tison).
Artocarpus.
Arundo.
Asarum.
Banisteria.
Begonia.
Betula.
Bigonia.
Boehmeria.
Broussonetia.
Byronia.
Canna.
Carica.
Carya.
Cassis.
Castanea.
Catalpa.
Celtis.
Chiliantus.
Cladrastis (Tison).
Coccoloba.
Coleus.
Convolvulus (Dutailly).
Cornus (Maüle).
Corypha.
Cucumis.
Cucurbita.
Cuspidaria.
Dahlia.
Diospyros.
Elaeagnus.
Euphorbia.
Fagus.
Ficus.
Fraxinus.
Gleditsia (Tison).
Hammamelis (Tison).
Hedera.
Hedychuim.
Heliconia.
Humulus (Tubeuf).
Inula.
Jatropha.
Juglans.
Koelreuteria.
Latania.
Laurus.
Ligustrum.
Loranthus.
Loxapteryguim.
Machura.
Mansoa.
Maranta.
Micania.
Morus.
Musa.
Ochroma.
Olea.
Ostrya.
Passiflora.
Paulownia.
Perilla.
Pharbitis.
Philodendron.
Phyllanthus.
Picea (Raatz).
Pinus (Raatz).
Piratinera.
Pistacia.
Plantago.
Platanus.
Populus.
Portulacca.
Prunus (Wieler).
Pterocarya.
Quercus.
Rhus.
Ricinus.
Robinia.
Rosa (Maüle).
Rubia.
Rumex (Dutailly).
Salix.
Sambucus.
Santalum.
Schinus.
Sideroxylum.
Solanum.
Sparmannia.
Strelitzia.
Styinatophyllum.
Taraxacum.
Thunbergia.
Ulmus.
Urtica.
Vitis.
Xanthoxylon (Tison).

[2] The cross section of a mature tree may be divided into at least two regions: The outer or last-formed rings, variable in number, which are termed the "sapwood" or "alburnum," and the inner rings around the pith or center of the tree, which in dry material are sometimes indistinguishable in appearance from sapwood, but which are more often definitely marked by a difference in color and are then termed the "heartwood" or "duramen." (Pl. LIX, fig. 2.)

found to check reasonably well, as shown in Table II. The greatest variation occurs in the species in which tyloses are very rare or else scatteringly developed and, therefore, where their practical importance is relatively slight.

OCCURRENCE OF TYLOSES IN NATIVE HARDWOODS

Table II gives the results of observations made on the distribution and region of first development of tyloses in 143 specimens of hardwoods grown in the United States. The very marked development of tyloses in certain species has been noted in Table I.

Special attention was given to the early development of tyloses. The results show their presence in the sapwood of all the species in which they occur in the heartwood. The hickories, for instance, give some interesting data concerning the occurrence of tyloses in sapwood. It has been maintained that if tyloses ever occurred in sapwood they would be found only in very narrow sapwood—that is, where the transition from sap to heartwood begins at the end of the first or second year after the ring is formed, as, for instance, in some of the oaks. In the hickories, however, tyloses are always present in the sapwood, and are generally developed even in the outermost rings as abundantly as in the heartwood. Plate LIII, figure 3, shows a cross section of the sapwood of pignut hickory (*Hicoria glabra*), including the fourth to the seventh rings in from the bark. This particular tree had 31 rings of sap, or uncolored wood, and tyloses were well developed in the very outermost rings. (Pl. LIX, fig. 1.)

Tyloses are normally lacking in the red-oak group, although there are many exceptions. An illustration of vessels not filled by tyloses is given by those in the middle of Plate LIV, *R*2, and by some of those in Plate LV, figure 1. In some cases tyloses occur in individual vessels in species ordinarily free from them, as Spanish oak. (Table II.) In several instances the few scattered tyloses present in both the sapwood and heartwood have a rather abnormal appearance and are associated with areas of fungous growth. (Table II, Scarlet oak.) In certain species of the red-oak group, however, as blackjack oak (*Quercus marilandica*), tyloses are very generally developed in both the sapwood and heartwood.

In the white oaks, in contrast to the red-oak group, tyloses are generally very abundant, even in the outermost rings. Some of the white oaks where tyloses are slow in forming show striking examples of the growth and development of the tylose in its early stages. This is illustrated in Plate LII, figure 3, which is a reproduction of a photomicrograph of a cross section of California white oak, or valley oak (*Quercus lobata*), showing a piece of the sapwood next to the bark. Fragments of the bark may be seen at the top of the illustration. The relatively small bladderlike cells here shown increase in size until they grow together and fill the vessels as shown at the bottom of this illustration and in Plates LIII, figures 1, 2, and 3, and LV, figure 2.

TABLE II.—*Occurrence of tyloses in the large vessels of the hardwoods.*[1]

THE DIFFUSE POROUS WOODS.

Species.	Common name.	Tyloses in sapwood.			Tyloses in heartwood.
		Counting in from bark—		General character.	
		First appear in ring—	Full development in ring—		
Acer macrophyllum Pursh	Oregon maple			None present	None present ("gum" frequently found).
Acer negundo L.	Box elder			do	Do.
Acer rubrum L.	Red or soft maple			do	Do.
Acer saccharinum L.	Silver maple			do	Do.
Acer saccharum Marsh.	Sugar or hard maple			do	Do.
Acer saccharum nigrum (Michx. f.) Britt.	Black maple			do	None present ("gum" abundant).
Aesculus octandra Marsh.	Yellow buckeye			Very rare, scattered (Pl. LII, fig. 2).	Scattered (most near pith).
Ailanthus glandulosa Desf.	Tree of heaven			None present	None present ("gum" frequently found).
Alnus oregona Nutt.	Red alder			No material	Do.
Amelanchier [2] canadensis (L.) Medic.	Service			None present	None present.
Betula lenta L.	Sweet birch			do	None present ("gum" frequently found).
Betula lutea Michx. f.	Yellow birch			do	Do.
Betula nigra L.	River birch			do	None present ("gum" sometimes found).
Betula papyrifera Marsh.	Paper birch			do	Do.
Betula populifolia Marsh.	White birch			do	Do.
Carpinus caroliniana	Blue beech			do	None present.
Cornus florida L.	Flowering dogwood			do	Do.
Do	do			do	Do.
Do	do			do	Do.
Crataegus [2] coccinea L.	Scarlet haw			None present. (Rare exceptions near wounds.)	Same as in sapwood ("gum" abundant).
Crataegus tomentosa L.	Pear haw			None present	Do.
Fagus atropunicea (Marsh.) Sudw.	Beech whiteheart			Very rare	Same as in sapwood.
Do	Beech with red heartwood			Few	Many vessels generally filled.
Do	do			Very rare	Do.

Hamamelis virginiana L.	Witch-hazel			None present	None present.
Ilex opaca Ait.	American holly			do	Do.
Kalmia latifolia L.	Mountain laurel			do	Do.
Liquidambar styraciflua L.	Red gum			Scattered; a few well-developed examples.	Scattered.
Do	do			Generally scattered throughout	Same as in sapwood.
Do	do			No material	Generally scattered throughout.
Liriodendron tulipifera L.	Yellow poplar			Scattered	Do.
Do	do			No material	Few scattered.
Magnolia acuminata L.	Cucumber tree			Scattered; frequent	Scattered; frequent.
Magnolia fraseri Walt.	Fraser umbrella			Very rare; scattered	Do.
Magnolia glauca L.	Sweet magnolia			Rare; scattered	Do.
Mohrodendron carolinum (L.) Britt.	Silverbell tree			None present	None present.
Nyssa sylvatica Marsh.	Black gum			do	Do.
Nyssa aquatica L.	Water gum			do	Do.
Oxydendrum arboreum	Sourwood			Few	Scattered (numerous near pith).
Platanus occidentalis L.	Sycamore			Scattered	Scattered.
Do	do			Very rare	Rare (almost entirely lacking except near pith).
Do	do			No material	Frequent; scattered.
Populus grandidentata Michx.	Large-tooth aspen			Numerous	Many.
Populus tremuloides Michx.	Aspen	2		Scattered	Same as in sapwood (many near pith).
Do	do	1		Scattered (many in two outer rings).	Scattered.
Do	do			No material	Scattered; numerous.
Populus trichocarpa Torr. and Gr.	Black cottonwood			Scattered	Scattered.
Do	do			No material	Few.
Prunus serotina Ehrh.	Black cherry			None present	None present (abundant gum).
Do	do			do	Do.
Prunus pennsylvanica L. f.	Wild red cherry			do	Do.
Rhododendron maximum L.	Great rhododendron			do	None present.
Salix nigra Marsh.	Black willow	1		Numerous in four outer rings; scattered throughout.	Scattered.
Do	do			Very rare	Very rare except in two rings near pith.
Tilia americana L.	Basswood			None present	None present.
Do	do			No material	Do.

[1] Both the Latin and common names used are those given by G. B. Sudworth in Bulletin 17, Division of Forestry, Department of Agriculture, 1898, and in a later unpublished revision of the same.

[2] One of the Rosaceae, tyloses generally lacking in this family (Molisch).

TABLE II.—*Occurrence of tyloses in the large vessels of the hardwoods*—Continued.

THE RING POROUS WOODS.

Species.	Common name.	Tyloses in sapwood.			Tyloses in heartwood.
		Counting in from bark—		General character.	
		First appear in ring—	Full development in ring—		
Castanea dentata (Marsh.) Borkh	Chestnut	1	4	Numerous; not exceedingly abundant.	Present throughout (rather thin walled).
Do	do			No material	Abundant.
Catalpa speciosa Warder	Hardy catalpa	1	2	Abundant; thin walled	Abundant throughout.
Celtis occidentalis L	Hackberry	Outer rings		Scattered; well developed	Numerous; slightly more than in sapwood.
Do	do			No sapwood material	Numerous.
Cercidium torreyanum (Wats.) Sarg	Palo Verde			None present	None present (abundant gum).
Do	do			do	Do.
Chilopsis linearis (Cav.) Sweet	Desert willow	1	3 on	Abundant; thin walled	Abundant throughout.
Diospyros virginiana L	Persimmon			None present	None present.
Eucalyptus globulus Lab	Blue gum			Scattered; few	Scattered.
Fraxinus americana L	White ash	1	2 on	Scattered; numerous; very thin-walled.	Numerous; same as in sapwood.
Do	do	2	do	Numerous; very thin-walled	Numerous (vessels generally filled).
Do	do			No material	Do.
Fraxinus lanceolata Borkh	Green ash	1	2 on	Abundant; very thin-walled	Same as in sapwood.
Fraxinus nigra Marsh	Black ash			None present (much fungus in specimen).	None present (some "gum" present).
Fraxinus oregona Nutt	Oregon ash	1		Very few; exceptionally; cases due to wounding in outermost ring.	Very few; thin; poorly developed.
Do	do			Outer sap lacking; many well developed but thin-walled.	No material.
Fraxinus profunda Bush	Pumpkin ash	1	2 on	Abundant; very thin-walled	Same as in sapwood.
Fraxinus quadrangulata Michx	Blue ash	1	do	do	Do.
Gleditsia triacanthos L	Honey locust			None present	None present (gum frequently found).

Gymnocladus dioicus (L.) Koch	Coffee tree				
Hicoria alba (L.) Britt	Mockernut (hickory)	2	3 on	Generally well developed	Throughout as in sapwood.
Do	do	2	do	do	Do.
Do	do	1 to 4 few	5 on	do	Do.
Do	do	1 to 2 few	3 on	do	Do.
Do	do			No material	Throughout.
Hicoria aquatica (Michx. f.) Britt	Water hickory	1	8 on	Generally well developed	Do.
Hicoria glabra (Mill.) Britt	Pignut (hickory)	1	2 on	Abundant throughout	Same as in sapwood.
Do	do	2 to 3 few	4 on	do	Do.
Do	do	1	2 on	do	Do.
Do	do	1 to 3 least	4 on	do	Do.
Do	do	1 to 4 least	5 on	Abundant throughout (exceptionally wide sapwood).	Do.
Hicoria laciniosa (Michx. f.) Sarg	Shellback (hickory)	1 to 3 least	4 on	Abundant throughout	Do.
Do	do	do	do	do	Do.
Hicoria minima (Marsh.) Britt	Bitternut	1	2 on	Many	Do.
Hicoria myristicaeformis (Michx. f.) Britt	Nutmeg (hickory)	1	do	Generally well developed	Do.
Hicoria odorata (Marsh.) Sarg	Small pignut (hickory)	2 to 3 few	3 on	do	Do.
Hicoria ovata (Mill.) Britt	Shagbark (hickory)	1	2 on	do	Do.
Do	do	1 to 3 least	4 on	do	Do.
Do	do	1	2 on	do	Do.
	Five miscellaneous hickories	Outer sapwood not furnished.		do	Do.
Juglans cinerea L	Butternut	1	2 on	Generally developed; many; most in 2 outer rings.	Many; full development.
Do	do	1	do	Generally developed; many; most in 7 outer rings.	Do.
Do	do			No material	Do.
Juglans nigra L	Black walnut	1	1 on	Abundant throughout	Less strongly developed than in sapwood.
Do	do			No material	Abundant.
Morus rubra L	Red mulberry	2	3 on	Very abundant	Same as in sapwood.
Prosopis juliflora (Swartz) de C	Mesquite			None present	None present (abundant "gum").
Erythrobalanus, the red oaks or black oaks:					
Quercus rubra L	Red oak			None present except as rare exceptions.	Same as in sapwood.
Do	Red oak (misc.)			do	Do.
Quercus coccinea Muenchh	Scarlet oak	Abnormally numerous in 2 outer rings.		Scattered in third and fourth rings; generally absent. Fungus present; tyloses have abnormal appearance.	Do.
Do	do			None present	None present.
Quercus digitata (Marsh.) Sudw	(Highland) Spanish oak			Very rare; one per ring to the inch	Same as in sapwood.
Quercus imbricaria Michx	Shingle oak			Scattered	Do.
Quercus marilandica Muenchh	Black jack	3	3 on	Strongly developed; full, large vessels.	Do.

TABLE II.—*Occurrence of tyloses in the large vessels of the hardwoods*—Continued.

THE RING POROUS WOODS—Continued.

Species.	Common name.	Tyloses in sapwood.			Tyloses in heartwood.
		Counting in from bark—		General character.	
		First appear in ring—	Full development in ring—		
Erythrobalanus the red oaks or black oaks—Continued.					
Quercus palustris Muenchh	Pin oak			Extremely rare	Same as sapwood.
Do	do			None present	None present.
Quercus phellos L	Willow oak			Very rare	Very rare.
Quercus velutina Lam	Black or yellow oak	2	2 to 4 full	After fourth, scattered, frequent	Scattered.
Lepidobalanus, the white oaks:					
Quercus alba L	White oak	1	3 on	Very numerous, young forms in outer rings.	Well developed throughout.
Do	do	1	3 on	do	Do.
Do	do	1	3 on	Strong development	Abundant.
Do	do	2	2 on	do	Do.
Do	do			No material	Many.
Quercus garryana Dougl	Garry oak	2	2 on	Very abundant	Same as in sapwood.
Do	do			No material	Do.
Quercus lyrata Walt	Overcup oak			Many; generally developed	Do.
Quercus lobata Nee	Valley oak	1		Many; slow-forming, abundant young tyloses.	Abundant.
Quercus macrocarpa Michx	Bur oak	1	1	Numerous to many; somewhat irregular.	Do.
Quercus michauxii Nutt	Cow oak	1; many thin walled, 2; few or lacking.	3 on	Many	Do.
Quercus minor (Marsh.) Sarg	Post oak	1	3 on	Abundant general development	Do.
Quercus platanoides (Lam.) Sudw	Swamp white oak	1	3 on	Show young forms and gradual increase.	Do.
Quercus prinus L	Chestnut oak			Rare exceptions	Exceptions present.
Do	do			None present	None present.

Blotobalanus, the live oaks:					
Quercus densiflora Hook. and Arn	Tanbark oak			Scattered; thick walled	Same as in sapwood.
Do	do			Scattered	Abundant.
Quercus chrysolepis Liebm	Canyon live oak			Generally lacking	Same as in sapwood.
Rhus hirta (L.) Sudw	Shaghorn sumach	1	1 on	Abundant	Do.
Robinia pseudacacia L	Black locust	1	1 on	Abundant; all vessels crowded with thin-walled tyloses.	Do.
Do	do	1	2 on	Abundant	Do.
Sassafras sassafras (L.) Karst	Sassafras			Scattered; frequent	Do.
Toxylon pomeferum Raf	Osage orange	1	1 on	Abundant; vessels crowded with thin-walled tyloses.	Do.
Ulmus alata Michx	Wing elm			Numerous; scattered	Do.
Ulmus americana L	White elm			Rare	Do.
Do	do			Generally scattered	Do.
Do	do			Rare; few	Do.
Ulmus pubescens Walt	Slippery elm			Rare	Do.
Do	do			Few; scattered	Do.

TYLOSES IN SOFTWOODS

Coniferous or softwoods lack the large open pores or vessels which characterize the hardwoods. They also either lack or show a scanty development of wood parenchyma, the chief source of tylose formation in the hardwoods. Since it is in relation to the closing of the vessels that tyloses are of practical significance, the study of tylose distribution in the conifers is of relatively small importance. However, since tyloses or tyloselike cells are often present in the tracheids or in the resin canals of certain normal coniferous woods, and since they have been found to play some part in penetration of wood preservatives and in resin flow, their occurrence in the softwoods was studied.

The occurrence of tyloses in coniferous woods has not received the attention given to their occurrence in hardwoods. Often their presence has been ignored, or they have been reported as entirely lacking.[1] When studied, moreover, investigations were usually confined to parts of the plant other than the wood,[2] though there are a few notable observations on their occurrence in the wood itself (Boehm; Chrysler; Conwentz; Küster; Mayr; Penhallow; Raatz).

TRUE TYLOSES IN CONIFERS

Tyloses in normal coniferous wood arise chiefly from the parenchymatous cells of the medullary rays. (Pl. LVI, figs. 1 and 2.) As in the hardwoods, it is by the growth of the membranes of the one-sided bordered pits that tyloses are formed, especially where the pits are of large size, as in the white pines. In this case tyloses grow into the lumen of the tracheid, just as in hardwoods they grow into the vessels or pores. Tracheids, like vessels, function as sap conductors, but instead of having in their end walls actual openings of considerable size they have only relatively thin regions or pits. These are more or less completely closed by an irregularly thickened membrane, portions of which sometimes contain very minute perforations (Bailey). Thus in these elements already closed or nearly closed, tyloses have not the effect that they have in the open vessels of the hardwoods. Moreover, tylose formation of this type in conifers can only take place in a comparatively small percentage of the tracheids—that is, in those adjacent to the medullary-ray parenchyma cells produced as a result of wounds (Boehm; Raatz).[3]

TYLOSELIKE CELLS IN THE RESIN CANALS

Aside from true tyloses, there is often observed in certain species of conifers a partial or complete closing of the resin canals, produced by parenchyma cells, but not by growth of the membrane of the one-sided

[1] Reported by Molisch after examining 700 species of plants of all sorts.

[2] They are said to be more abundant in the root than in the stem (Raatz). They also have been studied in the leaf and in the cone axis.

[3] Boehm and Raatz observed tyloses as a result of wounding in *Abies pectinata*, *Pinus sylvestris*, *Pinus strobus*, *Pinus excelsa*, *Larix europea*, and *Thuja occidentalis*.

bordered pit. Such growths are termed "tyloselike," since they produce an effect very similar to that produced by the true tyloses of the hardwoods.

Resin canals or ducts are normally present in the following coniferous genera: Larch, or tamarack (Larix), spruce (Picea), Douglas fir (Pseudotsuga), and pine (Pinus). These canals when seen in cross section often bear a superficial resemblance to the vessels or pores of the hardwoods. (Pl. LVII, fig. 1.) They are, however, different in both their origin and function. Resin ducts are not cellular elements, but simply intercellular spaces which result from the splitting apart of the common walls of a group of parenchyma cells. A very early stage of this splitting is shown in Plate LVI, figure 1. These parenchyma cells which surround the canal opening are called "epithelial cells." They are the seat of resin formation, and they cause the tyloselike closing of the resin canal. Certain of them often remain thin walled and contain plasma. (Pl. LVIII, figs. 2 and 5.) After they split apart to form the canal, when they change in shape and size, a further swelling and growth may take place which closes the canal entirely or in part. (Pls. LVII, figs. 1 and 2, and LVIII, figs. 2, 5, and 6.) The fact that it is the growth or expansion of the whole cell, and not a portion of the wall of that cell, together with a portion of the wall of the neighboring cell, as in the tylose-forming membrane of the one-sided bordered pits of the hardwoods, clearly indicates the difference between the true tyloses of the hardwoods and the tyloselike cells in the resin canals of the conifers.

OCCURRENCE OF TYLOSES AND TYLOSELIKE CELLS IN NATIVE CONIFERS

Over 600 permanently mounted sections from coniferous woods in the collection of the Forest-Products Laboratory were specially studied, while more than three times this number were examined unmounted.

TRUE TYLOSES

Ray or true tyloses were found in the normal wood of the conifers, but were not abundant. Their shape and general appearance are well illustrated in Plate LVI, figures 1 and 2. None of the long, saclike vesicles which sometimes fill the whole tracheid lumen in the roots of conifers were found. The greatest development of true tyloses was found in the soft pines. In this group they were better developed in spring wood than in summer wood and were more numerous in the sapwood than in the heartwood. Indeed, some of the pit membranes in the heartwood were concave in shape, appearing to have collapsed inward instead of protruding into the tracheid.

The size of the pits between the medullary ray cells and the tracheids in conifers bears a definite relation to the formation of tyloses. As a rule, the ray pits in the hard pines are small and tyloses are lacking,

Norway pine (*Pinus resinosa*), which is regarded as a hard or pitch pine, offers an exception to this. Here we find numerous tyloses, but here also we have large ray pits. The only soft pine examined which did not contain tyloses was piñon pine (*Pinus edulis*). This species is characterized by small ray pits instead of the large ones common to this group.

Of the other conifers all of the species listed below have small ray pits. No true tyloses were found in these species. (See Table III.)

TABLE III.—*Occurrence of true tyloses in native conifers.*

Species.	Number of specimens.	Sapwood.	Heartwood.
SOFT PINES.			
Limber pine (*Pinus flexilis*)	1	Abundant	
Sugar pine (*Pinus lambertiana*)	1	do	Numerous.
Western white pine (*Pinus monticola*)	1	do	Do.
White pine (*Pinus strobus*)	2	Numerous	Do.
Piñon pine (*Pinus edulis*)	1	None	None.
HARD PINES.			
Norway pine (*Pinus resinosa*)	2	Numerous	Numerous.
Jack pine (*Pinus divaricata*)	1	None	None.
Shortleaf pine (*Pinus echinata*)	3	do	Do.
Spruce pine (*Pinus glabra*)	1	do	Do.
Lodgepole pine (*Pinus murrayana*)	1	do	Do.
Longleaf pine (*Pinus palustris*)	1	do	Do.
Western yellow pine (*Pinus ponderosa*)	2	do	Do.
Pitch pine (*Pinus rigida*)	1	do	Do.
Loblolly pine (*Pinus taeda*)	1	do	Do.
Scrub pine (*Pinus virginiana*)	1	do	Do.
Table-mountain pine (*Pinus pungens*)	1	do	Do.
OTHER CONIFERS.			
Tamarack (*Larix laricina*)	1	None	None.
Western larch (*Larix occidentalis*)	1	do	Do.
European larch (*Larix larix*)	1	do	Do.
White spruce (*Picea canadensis*)	2	do	Do.
Engelmann spruce (*Picea engelmanni*)	1	do	Do.
Black spruce (*Picea mariana*)	1	do	Do.
Red spruce (*Picea rubens*)	2	do	Do.
Sitka spruce (*Picea sitchensis*)	2	do	Do.
Douglas fir (*Pseudotsuga taxifolia*)	2	do	Do.
Balsam fir (*Abies balsamea*)	2	do	Do.
White fir (*Abies concolor*)	1	do	Do.
Lowland fir (*Abies grandis*)	2	do	Do.
Alpine fir (*Abies lasiocarpa*)	1	do	Do.
Red fir (*Abies magnifica*)	1	do	Do.
Noble fir (*Abies nobilis*)	2	do	Do.
Port Orford cedar (*Chamaecyparis lawsonia*)	2	do	Do.
Yellow cedar (*Chamaecyparis nootkatensis*)	1	do	Do.
California juniper (*Juniperus californica*)	1	do	Do.

TABLE III.—*Occurrence of true tyloses in native conifers*—Continued.

OTHER CONIFERS—Continued.

Species.	Number of specimens.	Sapwood.	Heartwood.
Western juniper (*Juniperus occidentalis*)	1	None	None.
Red cedar (*Juniperus virginiana*)	1	...do	Do.
Incense cedar (*Libocedrus decurrens*)	1	...do	Do.
Redwood (*Sequoia sempervirens*)	1	...do	Do.
Bigtree (*Sequoia washingtoniana*)	1	...do	Do.
Bald cypress (*Taxodium distichum*)	1	...do	Do.
Yew (*Taxus brevifolia*)	1	...do	Do.
Arborvitæ (*Thuja occidentalis*)	1	...do	Do.
Western red cedar (*Thuja plicata*)	1	...do	Do.
Eastern hemlock (*Tsuga canadensis*)	1	...do	Do.
Western hemlock (*Tsuga heterphylla*)	2	...do	Do.
Black hemlock (*Tsuga mertensiana*)	1	...do	Do.

TYLOSELIKE CELLS

The tyloselike epithelial cells which surround the resin canals were also carefully studied in Pinus, Larix, Picea, and Pseudotsuga. In these woods both the horizontal and vertical resin canals often contained distended cells which partly or sometimes completely filled the canal openings. (Pl. LVII, fig. 2; and Pl. LVIII, figs. 2, 5, and 6.) This closed condition of the vertical canals is particularly noticeable near the medullary rays. (Pl. LVI, fig. 1; and Pl. LVII, fig. 2.) The distended closing cells correspond to the plasma-containing cells described on page 446. (Pl. LVIII, figs. 2 and 5.) A large number of the canals were, however, entirely open.

In pines where many of the epithelial cells remain capable of growth, three types of conditions may be found in the canals.

(1) The canals of the sapwood, especially of the outermost ring, may not have yet opened—that is, the space which the canal will occupy may still be filled by the parenchyma cells which later form the epithelium. (Pl. LVI, fig. 1.)

(2) Many canals may be partly open. (Pl. LVII, fig. 1.) Frequently the cells surrounding the opening are somewhat contracted and collapsed; or, again, individual cells containing plasma may become distended, bow out into the open lumen of the canal, and thus assist in partially closing it.

(3) Canals in the heartwood as well as in the outer rings of the sapwood may be completely closed.[1] This may come about in two ways: First, the groups of parenchyma cells observed in the sapwood may

[1] Compare Thomson, R. B.

never have split apart to form a canal opening. This was demonstrated by the writer by means of serial sections following the course of a number of horizontal resin canals from the bark into the heartwood. Second, the canals once open may be closed completely by the growth of certain of the epithelial cells, as before explained. This closing is not produced by the equal action of all the cells which first split apart to form the canal, but only by the later growth of certain of these which possessed plasma and the growth potential for a longer period than their neighbors. (Pl. LVIII, fig. 5.)[1]

PRACTICAL SIGNIFICANCE OF TYLOSES

TYLOSES AS A NATURAL "FILLER"

A good instance of the part played by tyloses in the structure of wood is in the case of red oak and white oak. These two species have practically the same structure, yet the red oak can not be used for tight cooperage stock because the vessels are open tubes through which air or liquid can escape. (Pl. LIV, middle.) In white oak the vessels are completely closed by tyloses, as shown in Plate LIII, figures 1 and 2, or Plate LIV, R_3.

In cabinetmaker's parlance, tyloses behave to some extent like a natural "filler." On a radial-cut surface the large vessels in the spring wood of a red oak appear like hollow grooves, while those in the white oaks are partly filled by the network of the tylosal cells which catch and hold paint, for example. (Pl. LII, fig. 1; and Pl. LIII, fig. 2.)

TYLOSES A FACTOR IN DURABILITY

It is of interest to note the presence of tyloses (or sometimes of gums) in the large vessels of those hardwoods which are particularly valued for their durability. Many factors, such as the chemical composition of the wood, its rate of growth, and hardness, are, of course, important in determining durability, but the effect of tyloses should not be disregarded. Moreover the vigorous growth of parenchyma, which in some cases manifests itself by causing tylose formation and in others by producing tannins, essential oils, etc., appears to be a fundamental characteristic of naturally durable woods. White oak, in which tyloses are abundant, is, for example, more durable than red oak, in which they are almost wholly absent. The tylose walls present an added obstruction to the advance of fungous hyphæ and tend to make the vessels impenetrable to air and water. They are especially effective in woods that have been dried.

Although sapwood contains tyloses, it is usually less durable than heartwood. The latter fact, however, holds true also for woods without tyloses and can probably be explained by the condition of such materials

[1] The illustrations reproduced in Pl. LVIII of all conditions of open and closed horizontal resin canals were taken from sapwood material.

in the sapwood as starches, which undergo a transformation when the heartwood is formed.

The following tabulation of the "Relative durability of hardwoods," compiled from the results of experiments, indicate that tyloses are a factor in durability. The more durable species will be found, with a few exceptions, to contain many or very abundantly developed tyloses. (See Tables I and II.)

RELATIVE DURABILITY OF HARDWOODS [1]

Durable.

Black locust.	Chestnut.	White oak.	Cherry.
Catalpa.	Black walnut.	Post oak.	Persimmon.
Osage orange.	Live oak.	Black ash.	Slippery elm.
Mulberry.	Sassafras.	Honey locust.	Bur oak.

Fairly durable.

Yellow poplar.	Red oak.	Scarlet oak.	Butternut.
Red ash.			

Not durable.

Cottonwood.	Black oak.	Black gum.	Gray birch.
White elm.	Red birch.	Watergum.	Paper birch.
Red gum.	Beech.	Basswood.	Aspen.
Hard maple.	Hickory.	Buckeye.	Willow.
White ash.	Cucumber.	Sycamore.	

The results of tests on 30,160 fence posts [2] indicated the following untreated hardwoods, in order of their durability, as the most suitable: Osage orange, locust, mulberry, catalpa, certain oak (species not given), and black walnut. The length of life in service varied from 10 to 50 years.

Some observations [3] on the life of untreated hardwood railroad ties further confirm the relation between tyloses and durability. It must be borne in mind, however, that for this type of service hardness has been considered in judging durability. The list of woods, together with their life in years under traffic, is as follows:

Species.	Years of service.	Species.	Years of service.
Butternut	[4] Few.	Black walnut	9
Beech	Do.	Chestnut	5 to 10
Black, red, or yellow oak	4 to 5	Hickory	7 to 10
Post oak	6 to 8	Black locust	7 to 10
Sassafras	6 to 8	White oak	5 to 12
Chestnut oak	9	Mulberry	[5] Many.
Bur oak	9	Catalpa	Do.

[1] This list is offered to show the comparative durability of some American timbers. It is not presumed to obtain for all conditions.

[2] Crumley, J. J. The relative durability of post timbers. Ohio Agr. Expt. Sta. Bul. 219, p. 605-640, 10 pl. 1910.

[3] Tratman, E. E. R. Report on the use of metal railroad ties and on preservative processes and metal tie-plates for wooden ties. U. S. Dept. Agr., Div. For. Bul. 9, p. 216. 1894.

[4] Life not given.

[5] Little used.

A few exceptions are noticeable. Chestnut oak, for example, has very few tyloses, but is hard and strong. Butternut has many tyloses, but it is also much softer than the oaks. Hickory has many tyloses and is here considered as durable a wood as black walnut. This is contrary to observations of its durability by other investigators. The kind of beech used is not specified, but if it was "white-heart" beech tyloses were not present. The "red-heart" beech, which contains tyloses, is often reported as a very durable wood.

The following recent estimates are based on experience and actual inspection by the Forest-Products Laboratory of woods in service (Table IV):

TABLE IV.—*Life of untreated wood placed subject to decay.*

Untreated material.	Years.	Untreated material.	Years.
Tyloses abundant or many; well developed.		*Tyloses lacking or scattered; few or weakly developed*—Contd.	
Lumber:		Lumber—Continued.	
Chestnut	12	Maple	4
White oak	8	Birch	4
Posts:		Poplar	4
Locust	25	Cottonwood	4
Osage orange	40	Tupelo	4
Mulberry	20	Basswood	4
Catalpa	14	White-heart beech	4
Chestnut	10	Red gum	4
White oak	8	Sycamore	3
Ties:		Posts:	
Black locust	20	Red oak	5
White oak	8	Ash	5
Chestnut	7	Aspen	5
		Gum	3
Tyloses lacking or scattered; few or weakly developed.		Ties:	
		White-heart beech	4
		Birch	4
Lumber:		Maple	4
Elm	7	Red oak	4
Ash	5	Gum	3

TYLOSES A FACTOR IN CREOSOTE PENETRATION

EXPERIMENTS WITH HARDWOODS

The study of the effect of structure on the penetration of artificial preservatives, such as creosote, is a separate problem. Preliminary work has shown some interesting results concerning the treatment of certain tylose-filled hardwoods. A piece of air-dry black locust (*Robinia pseudacacia*), 9 by 1½ by 1 inch, was subjected to a thorough treatment with creosote in a treating cylinder. The piece contained sapwood and heartwood, the vessels of both of which were filled with tyloses. The stick when split open after treatment showed no penetration except a faint discoloration in the outer one-fourth inch of sap, which apparently did not extend to the tyloses filling the vessels, but was located only in a few scattered groups of fibers. The failure of the wood to absorb creosote

was not entirely due to the presence of tyloses, but the fact that the creosote did not penetrate the tylose-filled vessels is significant.

In a piece of desert willow (*Chilopsis linearis*), 4 by 1½ by 2 inches, treated with carbolineum, no penetration was visible in the heartwood except about one thirty-second of an inch near the surface. In the sapwood, however, where, as shown in Table I, the large vessels of the two outer growth rings are without tyloses, the dark discoloration of the preservative was clearly visible following the lines of these open vessels.

Sapwood in general absorbs creosote much more easily than heartwood. The supposed absence of tyloses in this region of the tree has previously been regarded as one reason for this fact. As soon, therefore, as it was satisfactorily determined that tyloses were unmistakably present in the sapwood, special experiments were undertaken to discover what effect they had on the absorption of the creosote. A piece of white oak was given a commercial treatment at the same time and under the same conditions as the black locust. The sapwood absorbed the oil fully, but the penetration stopped abruptly at the line of color demarkation between the sapwood and heartwood. (Pl. LIX, fig. 2, *B*.) To the eye the heartwood, except for a surface coating, was absolutely untreated. The vessels in both the sapwood and heartwood of this piece were filled with strongly developed tyloses. Microscopic examination showed that the tyloses in the vessels of the treated sapwood were entirely uncolored and exactly like those in the vessels of the heart which was untreated throughout. The tyloses had then effectually kept the creosote out of the vessels, although there had been a full treatment of the wood fibers of the sapwood. This shows that a considerable quantity of the preservative was absorbed in spite of the fact that the presence of tyloses kept the creosote out of the vessels. Hence, tyloses of themselves need not be regarded as preventing the possibility of treating this species, at least in the sapwood.

A piece of oven-dried hickory, 2½ by 2½ by 14 inches, made up of both heartwood and sapwood, was treated at the same time and under the same conditions as the oak and locust, and showed a thoroughly good penetration throughout. (Pl. LIX, fig. 2, *C*.) Nevertheless, when the wood was split, the tyloses, which were abundantly developed in the vessels of both the sapwood and heartwood, were white and unstained by the creosote, showing a marked contrast to the dark-brown fibers of the surrounding treated wood. (Pl. LII, fig. 1.)

The preliminary observations just described concerning the penetration of creosote were based on results of treatments made on single specimens of the species studied and were regarded rather as valuable indications than as conclusive evidence. To check them with other results, the treatments with creosote were repeated on other specimens of the woods previously used and more specimens of another species containing many tyloses. First, a piece of hickory taken from miscellaneous material was given a high-pressure treatment with creosote.

A good absorption was obtained in both the sapwood and heartwood. Nevertheless, the tyloses, which were everywhere well developed and undamaged in the large vessels of both regions, remained colorless and untreated. In addition, two other blocks of hickory from material collected with special care were also given pressure treatments in the cylinder. These specimens were from pignut hickory, *Hicoria glabra*, and mockernut hickory, *Hicoria alba*. Both specimens contained sapwood and heartwood, with tyloses strongly developed in the large vessels. Again, the wood was thoroughly treated with creosote in both the sapwood and the heartwood, and once more the tyloses could be observed on a split surface to be quite uncolored and visible even to the naked eye through their marked contrast with the blackish brown of the treated wood. (Pl. LII, fig. 1.)

Thus, results from four specimens of hickory from different sources clearly showed that in spite of the presence of tyloses a high absorption of creosote may be obtained in the wood substance outside of the vessels and the tyloses filling them.

The other species used in these experiments was the so-called red-heart beech, a form of *Fagus atropunicea*. This had white tylose-free sapwood, but a reddish heartwood with many tyloses. It was treated in the cylinder at the same time as some of the hickories. The sapwood was thoroughly penetrated, but the heartwood remained untreated except for a surface coating and a very slight infiltration near the ends.

Lastly, a second piece of white oak was treated, as a check on the piece treated previously. After the creosote treatment, which was given at the same time as that of the hickories and beech, the sapwood was found to be penetrated, and, as before, the heartwood was unpenetrated. Careful examination showed, however, that the discoloration of the creosote extended down the large vessels of the sapwood and into the tyloses which they contained. This apparent contradiction of previous observations was explained when the material was examined under the microscope. The tyloses were found to be full of fungous mycelium and riddled with holes produced by the hyphæ in passing through the tylose walls. Under these circumstances, even when abundant tyloses are present, it is clear that some penetration may be secured in the vessels.

The marked difference to be observed in the penetrance of creosote in treatments of red oak and white oak is, however, chiefly the result of the presence or absence of tyloses. The unobstructed vessels of red oak give such open channels and offer so much additional surface for absorption through their walls that the penetrability of the other elements lying between the vessels is of relatively little importance. In white oak, on the other hand, it is only the elements of structure other than the large vessels that are available for penetration. The type of penetrance obtained in red oak is shown in Plate LIX, figure 2, *A*. The dark streaks mark the course of the creosote, which passed almost entirely

through the open vessels. The practical effect of this is evident in the results obtained in penetrance treatments. It is possible to force creosote for long distances through red oak just as it would be possible to force it through similar distances in small open pipe lines. In comparison with this, the distance the oil will pass through white oak is very short, since it has to penetrate through many cell walls, and the resistance of the material must be overcome by high pressures.

Thus, although tyloses have a distinct effect, they are not the only factor in the penetrance of wood. The characteristics of the other elements in the annual ring must be considered. However, in the cases examined, wherever the large vessels contained abundantly developed tyloses or filling cells, the vessels and the tyloses, but not necessarily the rest of the woody tissues, were impenetrable to creosote.

OBSERVATIONS ON CONIFERS

The presence of resin canals and their condition—that is, whether they are open or partly or entirely closed by cells—considered in conjunction with the general permeability of the tracheids, is a factor of practical significance in the selection of wood for creosoting. (Pls. LVI and LVII.) The number of the resin canals is very small in comparison with the number of tracheids. However, if the canals are unobstructed, penetrance is easily obtained for considerable distances through their cavities. In a wood whose tracheids are penetrated with difficulty, the creosote does not spread to any great extent from the canals into the tracheids, even when the former are full. Nevertheless, the presence of creosote or other toxic liquid in the resin-canal regions, which are among the first affected by fungous infection, is of considerable assistance in prolonging the life of the wood. Many of the resin canals, especially the vertical canals in both the sapwood and the heartwood of the pines, are not completely closed (Pl. LVII, fig. 1, and Pl. LVIII, figs. 1 and 4) and can for this reason be penetrated. The effect of the presence or absence of tylose-like cells in the resin canals, while a minor factor, is significant in connection with the treatment of poles, ties, and paving blocks.

EFFECT OF TYLOSES ON THE WATER-LOGGING OF WOOD

In order to test the effect of tyloses on the water-logging of wood, some roughly comparable air-dry blocks of several species were placed in a tank of water and the length of time required to water-log each block sufficiently to sink it was noted. The blocks were grouped with reference to their specific gravity (dry)[1] and their actual weight. The woods in which tyloses were few or wholly lacking invariably sank before those containing abundant tyloses. Chestnut oak sank before white oak and bur oak, persimmon before osage orange, flowering dogwood before hickory, yellow poplar and aspen before catalpa, and blue beech and honey locust

[1] Sargent, C. S. Report on the Forests of North America . . . 612 p., maps. Washington, 1884. (U. S. [10th Census Reports, v. 9].)

before black locust. The dogwood and persimmon sank in about 18 hours, while the catalpa floated for 20 days, and one piece of black locust with a large percentage of heartwood remained floating for 46 days.

SUMMARY

The 143 specimens of hardwoods examined included 45 genera (94 species), of which 24 contained tyloses. The 60 specimens of conifers examined included 13 genera (46 species), of which 1 contained tyloses. Of the 139 species examined, 56, belonging to 25 genera, contained tyloses.

Tyloses were found in the sapwood of all species in which they occurred in the heartwood.

Well-developed tyloses were found in the outermost rings near the bark of 30 species of hardwoods.

True tyloses occur in the wood tracheids of certain pines, principally of the white-pine group.

Epithelial cells sometimes effect a partial or even complete tyloselike closing of the resin canals in Pinus, Larix, Picea, and Pseudotsuga.

A considerable proportion of the vertical canals, even in the heartwood of the pines, are fully or partly open.

Tyloses act like a natural filler in the hardwoods.

The woods in which tyloses are abundant as a rule are durable.

Tyloses, because they are very impermeable to air, water, and creosote, reduce the penetrance of the woods in which they are strongly developed. The presence of tyloses in the vessels of a hardwood, however, does not prevent the penetrance of creosote into the other wood elements.

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PLATE LII

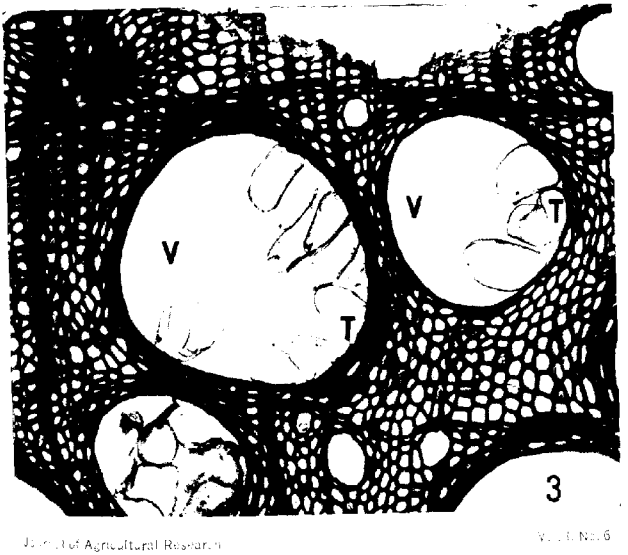
Fig. 1.—Split radial face of a creosoted hickory block, showing tyloses (*T*) in a large vessel. Magnified 12 diameters. Tyloses uncolored; remaining wood substance black with creosote.

Fig. 2.—Tangential section of *Aesculus octandra*, yellow buckeye × 680, showing two tyloses (*T*) which have grown out of one medullary-ray parenchyma cell (*MR*). Shows open connection between the tyloses and parenchyma cell.

Fig. 3.—Cross section of valley oak, a white oak, showing young tyloses (*T*) next the bark (*B*) in vessels (*V*).


T
1


MR
2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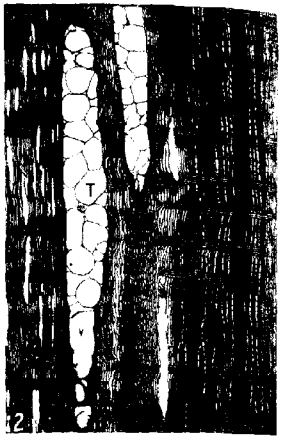
3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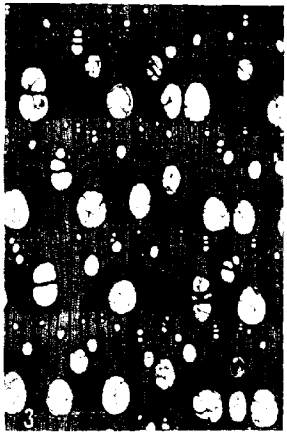

3


G
V
4

PLATE LIII

Fig. 1.—Cross section of a white oak, showing fully developed tyloses (*T*) in the large vessels (*V*).

Fig. 2.—Radial-longitudinal view, quarter-sawed surface, of the white oak shown in figure 1, showing complete closing of the vessel (*V*), which makes this wood valuable in light cooperage, etc.

Fig. 3.—Cross section of sapwood of pignut hickory, showing fully developed tyloses (*T*).

Fig. 4.—Radial view of mesquite, showing "gum" droplets (*G*) and formations often stimulating tyloses.

PLATE LIV

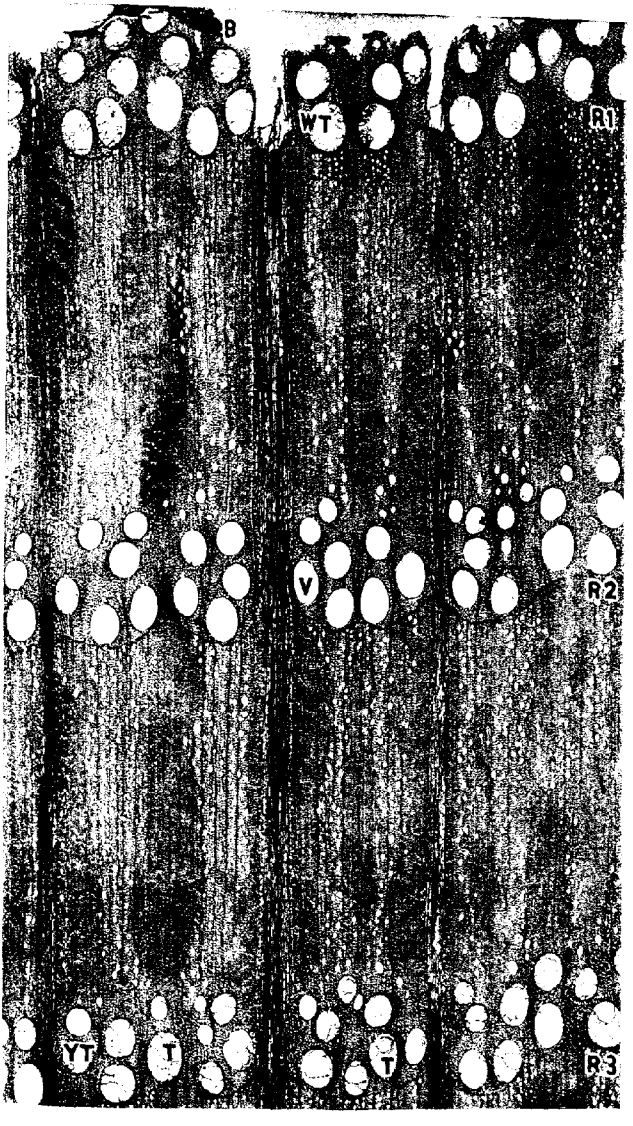
Cross section of cow oak, a white oak, showing normal and abnormal tyloses. From top to bottom are bark (*B*) and three annual growth rings (R_1, R_2, R_3).

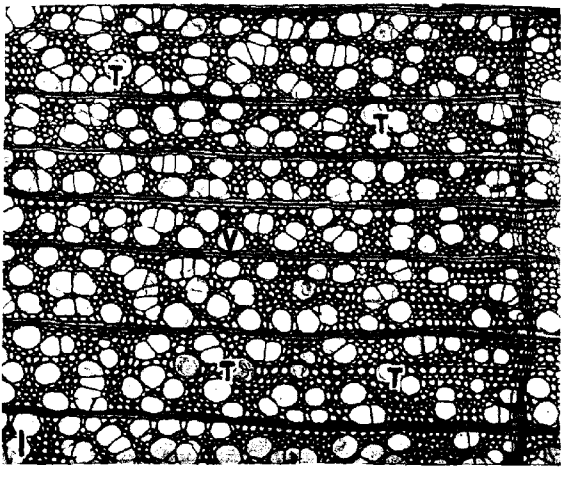
Fig. 1.—Wound tyloses (*WT*) induced by the felling of the tree and the sudden cessation of sap flow.

Fig. 2.—No tyloses (*V*); empty vessels. Normal tyloses not yet developed.

Fig. 3.—Young (*YT*) and well-developed normal tyloses (*T*).

PLATE LIV

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T
1

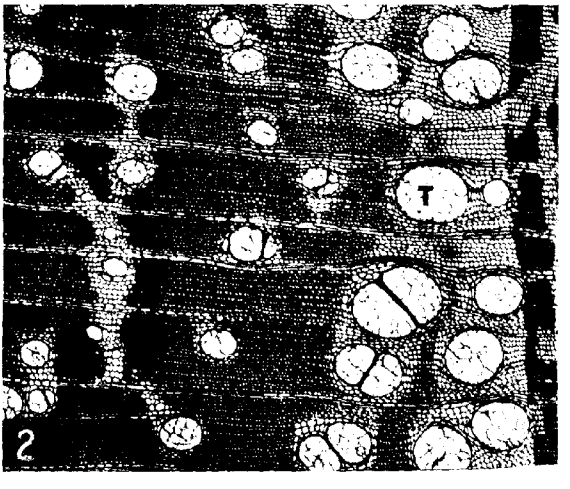

T
2

PLATE LV

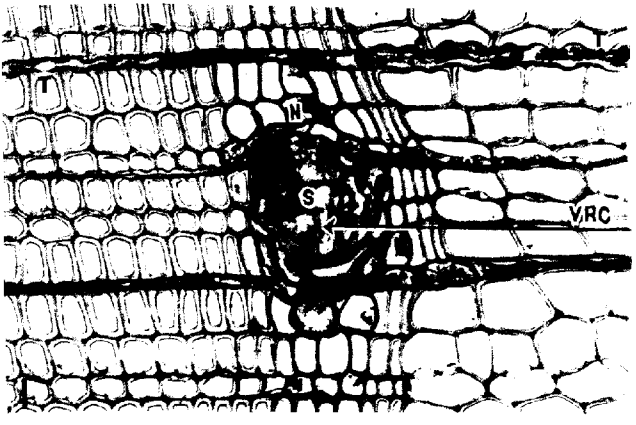
Fig. 1.—Cross section of a diffuse porous wood, yellow poplar or tulip, showing scattered tyloses × 50. *T*, tylose-filled vessels; *V*, empty vessels.

Fig. 2.—Cross section of a ring porous wood, osage orange, with vasicentric parenchyma, showing abundantly developed tyloses (*T*) × 50.

PLATE LVI

Fig. 1.—Cross section of western white pine, showing ray tyloses (*T*), closed vertical resin canal (*VRC*) in young sapwood, and nuclei (*N*) visible in epithelial cells of canal which is beginning to split open at *S*.

Fig. 2.—Tangential section of Norway pine, showing ray tyloses (*T*).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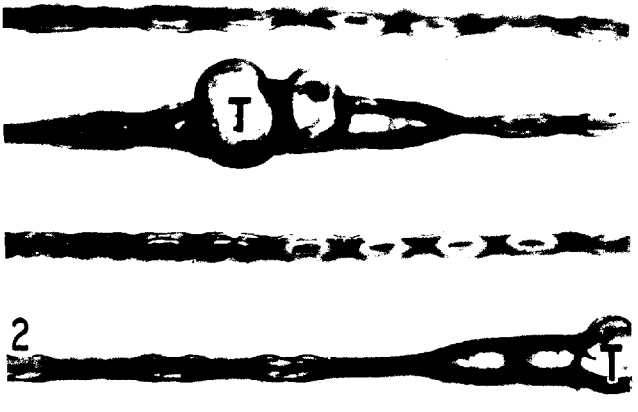
VRC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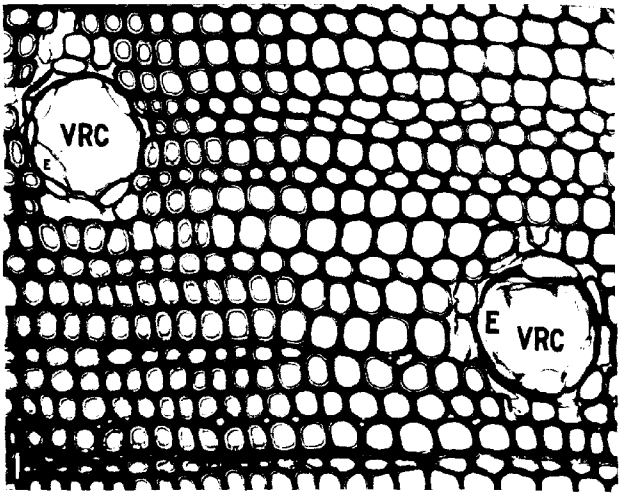

VRC
E
E
VRC

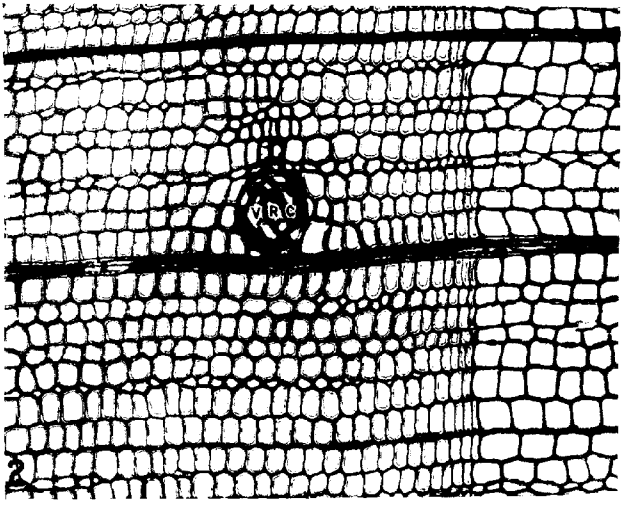

VRC

PLATE LVII

Fig. 1.—**Cross-section view** of shortleaf pine, showing open and partly closed vertical **resin canals** (*VRC*). These are typical of many canals in pine heartwood. Shows **thin-walled epithelial cells** (*E*).

Fig. 2.—**Heartwood of Sitka spruce**, showing closed vertical canal (*VRC*).

PLATE LVIII

Open and closed horizontal canals in sapwood.

Fig. 1.—Open canal in tamarack (*TE*) thick-walled epithelium.

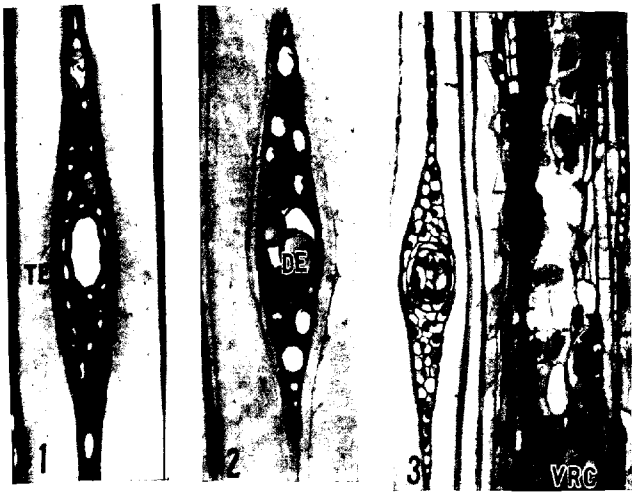
Fig. 2.—Partly closed canal with distended epithelial cells (*DE*) in Douglas fir.

Fig. 3.—Young canal which has never opened in western white pine. Cells with protoplasm and nuclei. Vertical canal (*VRC*) in same condition on right; this is longitudinal view of same canal as is shown in cross section, Plate LVI, figure 1.

Fig. 4.—Open canal in red spruce surrounded by thick-walled epithelium (*TE*).

Fig. 5.—Partly closed canal in red spruce. *TE*, thick-walled, and *DE*, thin-walled distended epithelial cells.

Fig. 6.—Closed canal in Engelmann spruce. From old sapwood. The epithelial cell has completely closed the canal and its wall has become thickened.


TE
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DE
2
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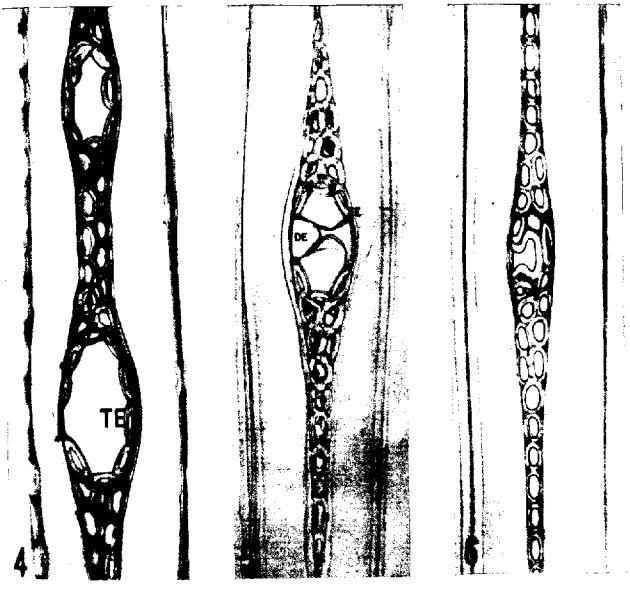
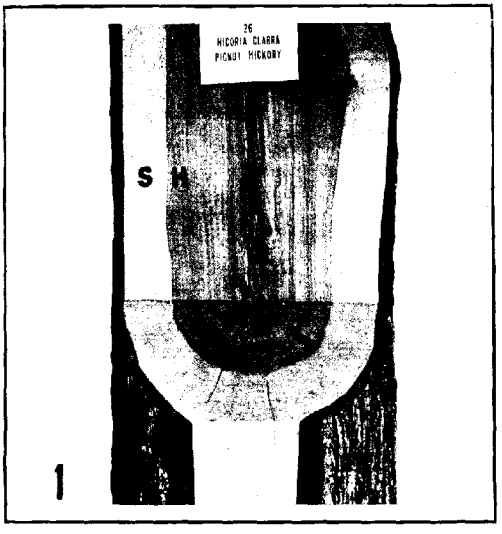
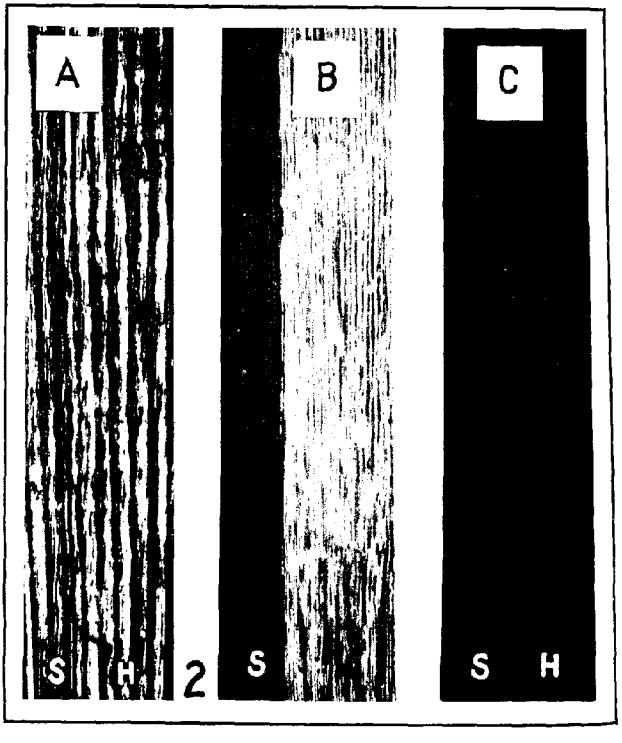
VRC


TE
4
DE


26
HICORIA CLARRA
PIGNUT HICKORY
S H
1


A
B
C
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2
S
S H

PLATE LIX

Fig. 1.—Log from collection of woods in the Forest-Products Laboratory—a specimen of the material used in this study; *S*, sapwood; *H*, heartwood.

Fig. 2.—Specimens of woods showing creosote penetrance in sap and heartwood as affected by tyloses. The three specimens each contain both sapwood and heartwood. Specimen *A*.—Red oak. Has no tyloses; creosote passed chiefly down the large vessels; note black streaks. Wood substance between vessels little treated; note white streaks. Specimen *B*.—White oak. Has abundant tyloses in sap and heartwood. Creosote penetrated the sapwood only. Thorough absorption obtained in the sapwood substance between the impenetrable, tylose-filled vessels. Specimen *C*.—Pignut hickory. Has abundant tyloses in sap and heartwood. Creosote penetrated both. Good absorption throughout in the wood substance between the tylose-filled vessels. Compare Plate LII, figure 1, an enlarged view of a portion of this block.

28736°—14——3.

THE CAMBIUM MINER IN RIVER BIRCH

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Bureau of Entomology

The species of the family Agromyzidæ generally mine in the leaves and stems of various plants, while some mine in their roots. The species presented in this paper, *Agromyza pruinosa* Coq.,[1] is quite out of the ordinary in that it mines in the cambium of the living tree, the mine leaving a scar known as a "pith-ray fleck."[2] These flecks in the various kinds of wood have been known for many years to be the result of the work of insects, and extensive investigations have been carried on in Europe as well as in this country in order to determine the species causing the damage. Investigations in Europe have proved that at least the pith-ray fleck in birch may be accredited to *Agromyza carbonaria*,[3] which is closely related to the American species. The pith-ray flecks in birch in America have been studied carefully, and it has been decided that *Agromyza pruinosa* is at least one of the insects that produce flecks and is possibly the only one. *Agromyza pruinosa* taken from river birch has just been reared to maturity. This is the first record in America of the production of flecks in birch by a definitely known species. (Pl. LX, fig. 2.)

SEASONAL HISTORY

During July and the early part of August, 1912, the work of this dipterous larva was very common in river birch at the Chain Bridge, in the District of Columbia, every tree that was examined containing new work; but in 1913, in the same locality, only a few trees disclosed new work. A dipterous larva and similar work were found frequently in red maple (*Acer rubrum*), but not so commonly as in birch. In 1913 Mr. T. E. Snyder found in wild cherry (*Prunus* sp.) on the Virginia shore of the Potomac River at the Chain Bridge two larvæ which are identical with the larvæ of *Agromyza pruinosa* in the birch, except that they are only two-thirds as long, although to all appearances full grown. The work of this species in wild cherry is identical with that in red maple and black birch, but the mines are correspondingly smaller.

[1] Thanks are due to Mr. J. R. Malloch for assistance in determining the species.

[2] Brown, H. P. Pith-ray flecks in wood. U. S. Dept. Agr., Forest Serv., Circ. 215, 15 p., 6 pl. May 7, 1913.

[3] Nielsen, J. C. Zoologische Studien über die Markflecke. Zool. Jahrb., Abt. System., Geogr. u. Biol. Tiere, Bd. 23, Heft 6, p. 725–738, pl. 30. 1906.

CHARACTER OF TREES ATTACKED

The trees attacked are apparently healthy, and infested ones can not be detected by their outward appearance. The only way in which to detect the larva is to remove the bark and expose the cambium, where at a glance you can generally recognize the new galleries from the old ones, since new larval mines are only faintly darker than the living cambium; in fact, they are sometimes of a delicate pink color, whereas all the old work is generally dark brown. In Vilas and Oneida Counties, Wis., the trees in the vicinity of Tomahawk and Trout Lakes were carefully examined by Mr. S. A. Rohwer last fall (1913), and no evidence of the cambium miner was found in white birch (*Betula populifolia*), red oak (*Quercus rubra*), red maple (*Acer rubrum*), or sugar maple (*Acer saccharum*).

Pith-ray flecks were found in red oak (*Quercus rubra*) at Charter Oak, Pa., by Mr. T. E. Snyder and in mountain holly (*Ilex monticola*) at Endeavor, Pa., by Mr. F. C. Craighead, but the particular insect or insects causing them are not yet known.

LIFE HISTORY OF THE SPECIES

METHODS OF REARING

Numerous experiments were conducted while rearing this species. All the breeding jars were placed in a pasteboard box, which was put in an ordinary soap box lined and covered with about five thicknesses of newspaper. This box was kept outside during the winter in an inclosed shed. The frost penetrated all the protective coverings, but not so thoroughly as though the boxes had been completely exposed. Jars containing earth and sand gave the best results in these rearing experiments. From April 15 to May 12, 1913, six adults emerged. On May 1 a single adult which was reared from the larva emerged, a hymenopterous parasite emerging from another pupa case on May 13.

THE EGG

The writer unfortunately did not succeed in securing the egg of this species, but it is apparently deposited in the fork of two branches which are about 5 to 8 years old and near the top of the tree. From the shape of the ovipositor (Pl. LXI, fig. 4) the egg is more than likely deposited on the outside of the bark, as the mine, which has been traced from a twig to the base of the tree, a distance of 40 feet, starts from this point like a hair line and, increasing in width as it goes down the trunk, reaches a width of one-eighth of an inch at the base.

THE LARVA[1]

The larva (Pl. LXI, fig. 1) is white, opaque, and cylindrical, averaging from 20 to 25 mm. in length and 1 mm. in diameter. One larva, collected

[1] The larva of this species was discovered by Mr. H. P. Brown and was first shown to the writer by Mr. T. E. Snyder.

on June 19, 1913, was 30 mm. in length and 1 mm. in diameter. The hooklet is shiny black and chitinized, the exposed portion being more highly chitinized than the rest. The hooklet complete (cephalopharyngeal skeleton) dissected out is shown in Plate LXI, figure 1, *a*. Back of the large hooklet are two smaller toothlike processes, one on each side, the position of these being shown at *b*. The anterior spiracles at *c* and the posterior pair at *d* are a very pale yellow, and their position is shown in outline. At the caudal end of the larva are two padlike surfaces, very faintly raised from the surface of the body, reaching nearly around the circumference of the body and covered with numerous brown, hooklike hairs or bristles. Several stages of the larvæ were observed, and the only noticeable difference was in their size.

If the larva reaches the base of the tree before the time to pupate, it will turn and mine up the cambium for some distance; on one occasion the larva retreated for 6 feet, then returned, thus encircling the root, and followed it for 2 feet from the trunk. The exit hole is sometimes made on the side of the root, but generally it is on the underside, and the larva pupates immediately on emergence. The pupæ were found from one-half to one inch from the exit hole. A portion of river birch (*Betula nigra*) with the bark removed is shown in Plate LX, figure 1, to illustrate the larval mines, while figure 2 is part of a cross section showing the "pith-ray flecks" from above.

The only larva that was reared by the writer, and in fact the only one that reached maturity, was placed in a large vial July 30, 1912, with a piece of freshly cut river-birch bark, the inner surface of which was covered freely with fresh sap. A piece of gauze was placed over the opening of the vial. On August 6, 1912, at 8.30 a. m., the larva commenced pupation, first becoming rigid and then changing to deep yellow at both ends, while the central portion remained the natural white color. It was 25 mm. in length and 1 mm. in diameter, but by noon it had decreased to about 10 mm. in length and increased to 2 mm. in diameter. Both ends had changed to dark brown and were perfectly formed, as in the pupa, and the middle was a light yellowish. At 5 p. m. the pupa was perfectly formed and dark brown all over, its dimensions now being 5 mm. in length and 2 mm. in diameter. The larva pupated under the thin folds of the outer bark, as there was nothing else in the vial.

THE PUPA[1]

The pupa (Pl. LXI, fig. 2) is of the usual cylindrical type and dark reddish brown in color, averaging from 4 to 5 mm. in length by 2 mm. in diameter, and is formed by the shrinking of the larval skin. The anterior spiracles are slightly more prominent than the posterior pair.

[1] The pupa of the species was discovered and first shown to the writer by Mr. T. E. Snyder.

THE ADULT

The adult (Pl. LXI, figs. 3 and 4) of *Agromyza pruinosa* Coq.,[1] six specimens of which were reared by the writer in the spring of 1913, is closely related to *Agromyza carbonaria* Zett. of Europe. *Agromyza pruinosa* remains in the pupal stage in the ground during the winter and emerges from the pupa case in one of two ways: Either the end of the pupal case is pushed off completely, or emergence is accomplished by tearing the end of the pupal case into shreds. Of the six specimens just referred to five were males and one a female. This species of Agromyza is represented in the United States National Museum collection by Coquillett's type, a single male specimen (Catalogue No. 6659, U. S. National Museum). The writer's specimens agree perfectly with the type, except that they are very slightly larger.

The general appearance of the adult female corresponds to that of the male, with the exception that it is slightly more robust. The ovipositor is slightly over one-half of a millimeter in length, chitinized, and somewhat shiny on the sides and edges of the dorsal surface. It is slightly flattened and a little broader at the apex than at the base. On the dorsal surface is a granular space, rounded toward the base of the ovipositor.

The total length of the female is 4 mm., and of the male about 3 mm. The abdomen of the female is shown in figure 4 of Plate LXI.

In an adult that had just emerged from the pupal case, the eyes were brownish and the frons and face a pale yellow or orange color. The thorax was pale gray, the legs yellowish, and the wings opaque white, clearing to hyaline in about two hours. The abdomen was of a dull orange color, with a faint gray line along the edge of each segment. The whole insect assumed its natural color in two and a half hours.

A HYMENOPTEROUS PARASITE

On May 13, 1913, a hymenopterous parasite, *Sympha agromyzae* Rohwer[2] (Pl. LXI, fig. 5), issued from a pupa case of *Agromyza pruinosa* Coq. This parasite is nearly as large as its host. Apparently it deposits its egg within the egg of the host. The apparently normal dipterous larva mines down the tree trunk and enters the ground; the pupa is perfectly formed, outwardly exhibiting no signs of parasitism, but about the time the host should emerge the parasite issues instead. At maturity the end of the pupal case is pushed open by the parasite in the same manner as the host would do it.

[1] Coquillett, D. W. New acalyptrate Diptera from North America. Jour. N. Y. Ent. Soc., v. 10, No. 4, p. 177–191. Dec., 1902. "*Agromyza pruinosa*, sp. nov.," p. 189.

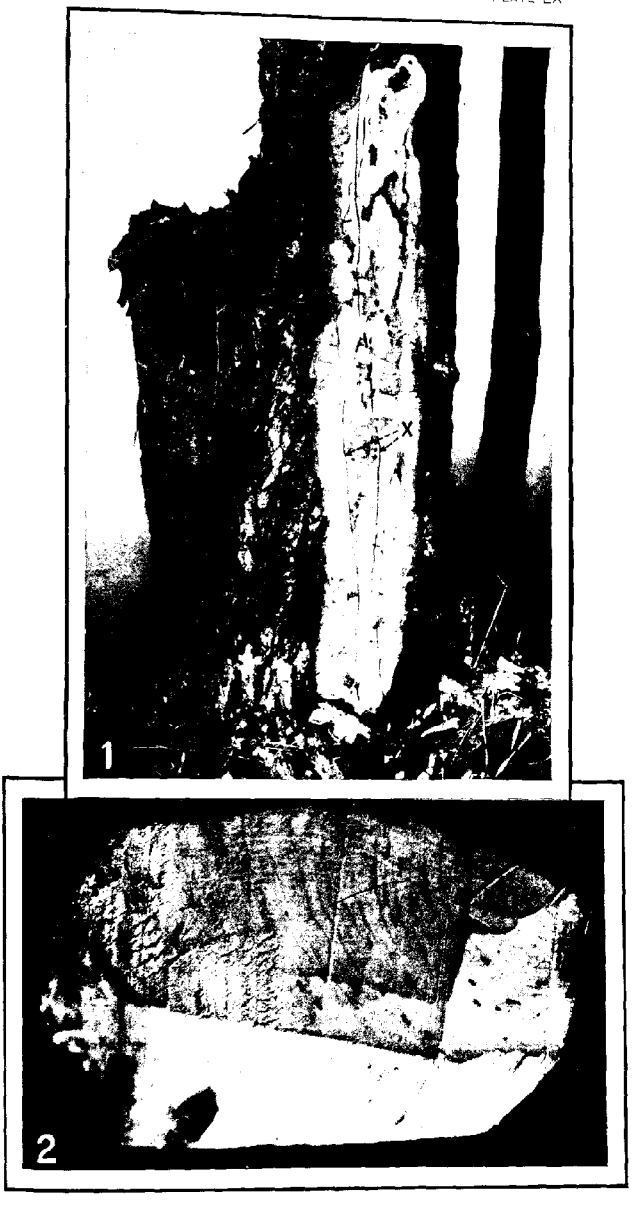
[2] "*Sympha agromyzae*, n. sp. Female. Length 3 mm. Notauli well defined; prescutum with a foveolate furrow; face sparsely punctured; propodeum with a transverse carina; hind tarsi pale. Type Cat. No. 16474 U. S. Nat. Mus." (S. A. Rohwer). A detailed description will appear later in the Entomological News.

PLATE LX

Fig. 1.—River birch with bark removed, showing larval mines of *Agromyza pruinosa*.

Fig. 2.—Section through wood of river birch, showing "pith-ray flecks" produced by the work of *Agromyza pruinosa*.

Photographed by H. B. Kirk.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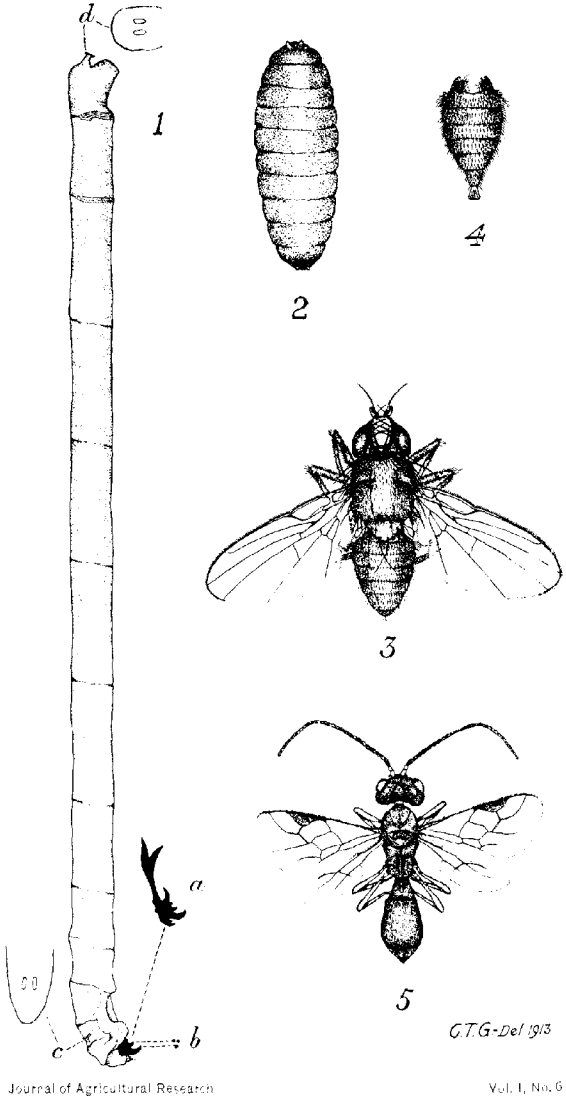
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PLATE LXI

Fig. 1.—*Agromyza pruinosa:* Larva and details.
Fig. 2.—*Agromyza pruinosa:* Pupa.
Fig. 3.—*Agromyza pruinosa:* Adult male.
Fig. 4.—*Agromyza pruinosa:* Abdomen of adult female, showing ovipositor.
Fig. 5.—*Sympha agromyzae:* Adult.

A STUDY OF SOME IMPERFECT FUNGI ISOLATED FROM WHEAT, OAT, AND BARLEY PLANTS

By Edward C. Johnson,
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INTRODUCTION

Of the imperfect fungi, many are parasitic on cereals wherever climatic conditions favor their development. They occur as scab on the heads, as leaf spots, and as infections in the culms and roots. Usually one or more species are present in the roots and culms of stunted plants, more particularly where some one cereal crop has been grown year after year on the same land. A study of the fungi occurring on wheat, oats, and barley, with particular reference to their pathogenicity, is therefore of much economic importance.

Such a study was begun in the cereal-disease laboratory of the Office of Grain Investigations of the Department of Agriculture in 1910. Species of imperfect fungi were isolated from wheat, oats, and barley obtained from various parts of the country. Helminthosporiums, Alternarias, Cladosporiums, and Fusariums were obtained. They were secured from leaf spots or from the lower nodes, root crowns, or roots of more or less stunted plants. In many cases they were obtained pure from fresh sporulating material on leaves and stems. In other cases they were obtained from the nodes, root crowns, and roots by sterilizing these parts externally in a 1 to 1,000 solution of mercuric chlorid, washing them in several changes of sterile water, and incubating them in moist chambers. After incubation for three to five days at a temperature of 72° to 77° F., sporulating myceliums were usually obtained. Plate cultures were then made and the fungi present isolated in pure cultures and propagated. On cornmeal agar, corn meal, and potato cylinders most of them grew and sporulated profusely.

Pure cultures were obtained and grown and the identity determined as *Fusarium culmorum* W. G. Sm., *Helminthosporium gramineum* Rabh., *Cladosporium gramineum* Cda., and a species of Alternaria. The determination of *Fusarium culmorum* was made by Dr. H. W. Wollenweber, of the Bureau of Plant Industry; the other determinations were made by the writer. *Helminthosporium gramineum* was isolated from the lower parts of the culms of stunted wheat plants growing on land continuously cropped to wheat at the Minnesota Agricultural Experiment Station, and from wheat leaves and barley leaves at the same

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place. *Cladosporium gramineum* was obtained from the leaves of oats at the same station. The Alternaria species occurring with Helminthosporium or independently were isolated from wheat culms in the same manner as *Helminthosporium gramineum*. *Fusarium culmorum* was isolated from wilted oat plants obtained from a 10-acre field on the farm of Mr. Peter Hanson, Sandy, Utah, on May 10, 1910. On this farm about 10 acres of oats had been practically destroyed by disease a few weeks after the seed was planted. The plants sent to the cereal-disease laboratory for examination and diagnosis were sterilized by immersing them in a 1 to 1,000 mercuric-chlorid solution for 10 minutes, followed by washing in sterile water. They were then placed in a moist chamber at a temperature of about 75° F. for several days and were soon covered with a luxuriant fungous growth. This proved to be a pure culture of *Fusarium culmorum*. It was plated and grown on potato cylinders and corn meal and sporulated abundantly.

After securing these fungi in pure cultures and inducing profuse sporulation, tests were made as to their pathogenicity on the leaves, seeds, and seedlings of wheat, oats, barley, and rye.

INOCULATION OF LEAVES OF WHEAT, OATS, BARLEY, AND RYE WITH SPECIES OF IMPERFECT FUNGI

Seedling plants of wheat (Haynes Bluestem, Minn. No. 169), oats (Early Gothland, Minn. No. 26), barley (Manchuria, Minn. No. 105), and rye (winter) were grown in the greenhouse at Washington, D. C., in 6-inch pots under temperature and moisture conditions as nearly normal as possible. When the seedlings were 2 to 3 inches high, inoculations were made about an inch from the leaf tip, with spores transferred from pure cultures in test tubes by means of a flattened inoculating needle, care being taken that little or none of the nutrient medium was transferred to the leaves. If any of the medium accompanied the spores, control plants were similarly treated with the same medium minus the spores. Care was taken not to injure the leaves in any way. The inoculated plants were placed under bell jars standing in pans of sand and water, thus permitting the moisture transpired to condense on the leaves, making an ideal condition for spore germination. They were allowed to remain under the bell jars for 48 hours and were then removed and placed in the greenhouse at a temperature ranging from 55° to 65° F. Table I shows the results of these inoculations.

TABLE I.—*Results of inoculating seedling leaves of wheat, barley, oats, and rye with imperfect fungi obtained from cereals.*

Test No.	Species.	Origin.	Inoculated on—	Date of inoculation.	Length of incubation.	Number of inoculations.	Infection.		Control.	
							Number.	Percentage.	Total number.	Number infected.
				1911.	*Days.*					
1	Helminthosporium gramineum.	Wheat node [1]	Wheat	Oct. 31	6	21	21	100	11	1
2	do	do	do	Nov. 10	4	33	17	51	12	0
3	do	do	Barley	Oct. 31	6	26	23	84	12	0
4	do	do	do	Nov. 10	4	50	39	78	12	0
5	do	do	Oats	Oct. 31	6	24	16	66	7	0
6	do	do	do	Nov. 10	4	25	6	24	12	0
7	do	do	Rye	do	4	36	8	22	14	0
				1912.						
8	do	Barley leaf [1]	Wheat	Jan. 24	5	43	40	93	15	0
9	do	do	Barley	do	5	67	67	100	25	0
10	do	do	Oats	do	5	50	50	100	20	0
11	do	do	Rye	do	5	45	45	100	22	0
12	Cladosporium gramineum.	Oat leaf [1]	Wheat	do	5	35	0			
13	do	do	do	do	6	47	0			
14	do	do	Barley	do	5	44	0			
15	do	do	do	do	6	64	0			
16	do	do	Oats	do	5	37	0			
17	do	do	do	do	6	108	0			
18	do	do	Rye	do	5	37	0			
19	do	do	do	do	6	32	0			
20	Fusarium culmorum.	Oat seedling [2]	Wheat	Mar. 19	14	50	0			
21	do	do	Barley	do	14	50	0			
22	do	do	Oats	do	14	50	0			
23	do	do	Rye	do	14	50	0			

[1] From University Farm, St. Paul, Minn. [2] From farm of Mr. Peter Hanson, Sandy, Utah.

Table I shows that the strains of *Helminthosporium gramineum* from both wheat and barley infected the leaves of wheat, barley, oats, and rye. On wheat, barley, and rye the leaf spots at the point of inoculation became distinct in a little less than three days after inoculation. These spots, which had a dead central area surrounded by a brown margin, slowly increased in size until their diameter was almost equal to the width of the leaf. A tendency to striation of the leaf area contiguous to the spots was noticed. No striking difference could be detected in the effect of the fungus from wheat or barley, the strain from wheat attacking barley and rye fully as severely as the strain from barley, and the strain from barley attacking wheat and rye fully as severely as the strain from wheat. On oats the two strains showed a slight difference in virulence, the fungus isolated from the barley apparently showing greater vigor in its attack than the fungus from wheat. In fact, three days after inoculation with the fungus from barley, oat leaves were so severely affected that in many cases they were cut in two, the tip portion often breaking off and falling to the ground. The two strains behaved so similarly, however, that physiologically they undoubtedly may be regarded as identical. Morphologically, no difference was detected.

Table I also shows that *Cladosporium gramineum* and *Fusarium culmorum* did not form leaf spots, even though the number of inoculated leaves was fairly large. This was rather unexpected in the case of Cladosporium, as it was obtained in pure culture by plating direct from a fresh mass of spores from a badly infected oat leaf in the field. Continuous culture on artificial media apparently either reduced its virulence, the temperature and moisture conditions in the greenhouse not being such as were conducive to infection by this fungus, or infection took place normally only after aphid injury or other wound. That *Fusarium culmorum* did not produce leaf spot was to be expected, as it usually does not occur in this manner and was not isolated from a leaf but from a wilted plant.

INOCULATION OF SEED OF WHEAT, OATS, BARLEY, AND RYE WITH SPECIES OF IMPERFECT FUNGI

Seed of wheat, oats, barley, and rye was inoculated with spores of the same strains of imperfect fungi used in the seedling-leaf inoculation tests. The fungi were grown in pure cultures in the same manner as those used for the leaf inoculation work. When sporulating profusely, sterile water was poured into the test tubes, the spore masses were loosened by the use of platinum needles, and the contents were well shaken. The water containing the spores was then poured off and diluted with sterile water until a drop placed under the microscope was found to contain from 5 to 25 or more spores. Seed of wheat, barley, oats, and rye was sterilized by immersion for one hour in a formalin solution consisting of 2.5 parts of 40 per cent formaldehyde to 1,000 parts of water and was immediately dried and inoculated with spores by soaking it in the water containing them. The seed was then planted in 6-inch pots filled with a sandy loam soil rich in humus and placed in the greenhouse at temperatures ranging from 55° to 65° F. The soil used had been sterilized previously in a steam sterilizer at a pressure of 15 pounds for two hours, the temperature being approximately 265° F. Control seed which had been sterilized but not inoculated was planted for comparison in every case. The results from such inoculation and plantings in the greenhouse are shown in Table II.

TABLE II.—*Results of inoculating seed of wheat, barley, and oats with imperfect fungi isolated from grain plants.*

Test No.	Species.	Origin.	Inoculated on—	Date of planting.	Inoculated seed.			Control seed.		
					Number planted.	Germinated.		Number planted.	Germinated.	
						Number.	Percentage.		Number.	Percentage.
				1911.						
1	Helminthosporium gramineum.	Wheat culm	Wheat	Nov. 21	150	34	22.6	90	68	75.5
2	do	do	do	Dec. 2	72	25	34.8	112	93	83.0
3	do	do	do	do	78	18	23.0	78	51	65.3
				1912.						
4	do	do	do	Jan. 19	105	64	60.9	105	92	87.6
				1911.						
5	do	do	do	Dec. 2	112	30	26.7	112	93	83.0
6	do	do	Barley	do	105	87	82.8	105	83	79.0
7	do	do	Oats	do	70	55	78.5	70	57	81.4
8	do	Barley leaf	Wheat	Nov. 19	105	62	59.0	105	92	87.6
9	do	do	Barley	do	105	86	81.9	105	83	79.0
10	do	do	Oats	do	70	52	74.2	70	57	81.4
				1912.						
11	Fusarium culmorum	Oat seedling	Wheat	Mar. 1	96	22	22.9	96	80	83.3
12	do	do	Barley	do	96	63	65.5	96	85	88.5
13	do	do	Oats	do	120	2	1.7	80	68	85.0
				1911.						
14	Alternaria sp	Wheat culm	Wheat	Nov. 21	150	108	61.2	90	73	81.1
15	do	do	do	Dec. 2	112	92	82.0	112	93	83.0
				1912.						
16	do	Wheat seedling	do	Mar. 5	80	79	98.7	80	75	93.7
17	do	do	Barley	do	80	69	86.2	72	62	86.1
18	do	do	Oats	do	90	77	85.5	90	79	87.7
19	Cladosporium gramineum.	Oat leaf	Wheat	Feb. 28	112	105	93.7	84	78	92.8
20	do	do	Barley	do	110	96	87.2	84	70	83.3
21	do	do	Oats	do	112	104	92.8	84	72	85.7

Table II shows that the strains of *Helminthosporium gramineum* isolated from wheat and barley were decidedly pathogenic to germinating wheat, only 22 to 60 per cent of the inoculated wheat in five trials producing plants, while 65 to 87 per cent of the controls not inoculated produced sound plants. These results are shown further in Plate LXII, figure 1. Barley and oats were not affected to any appreciable degree so far as germination and sprouting were concerned, the inoculated seed producing as large a percentage of plants as the clean seed. Those wheat plants which developed from inoculated seed were stunted and not nearly so vigorous as those produced from clean seed. At the end of six weeks the difference in height of plants from inoculated and clean seed was very marked. The plants from seed inoculated with *H. gramineum* from wheat were 5.5 inches high to the tip of the second leaf and those from seed inoculated with *H. gramineum* from barley 4.88 inches high to the tip of the second leaf, while control plants grown from clean seed averaged 6.45 inches high to the tip of the second leaf.

A similar difference was noticeable in barley plants grown from inoculated and clean seed, although the difference was not quite as marked

as in the wheat plants. This is shown in Plate LXII, figure 2. Barley plants from seed inoculated with *Helminthosporium gramineum* from barley were 5.82 inches high at the end of six weeks, those from seed inoculated with *H. gramineum* from wheat were 6.34 inches high, and those from clean seed, 6.46 inches high. The measurements are the averages of 50 plants in each case. There was no measurable difference in the height of oat plants grown from inoculated and from clean seed. *H. gramineum* was easily reisolated in every trial both from stunted wheat and stunted barley plants by external sterilization in mercuric-chlorid solution and incubation at room temperature.

Fusarium culmorum was even more virulent than *Helminthosporium gramineum*, particularly on oats. Inoculated wheat seed produced only 22.9 per cent of sound plants, barley seed 65.5 per cent, and oat seed only 1.7 per cent, while the controls produced 83.3, 88.5, and 85 per cent of sound plants, respectively. The results of the inoculations are further strikingly shown in Pl. LXII, figures 3, 4, and 5. The 10-acre oat field where this fungus was secured had been practically destroyed by some disease, and these results show that *F. culmorum* undoubtedly was the causal organism.

The two strains of *Alternaria* sp., one isolated from wheat culms from University Farm, St. Paul, Minn., the other from wilted wheat seedlings from Vermont, had no pathogenic effect on wheat, oats, or barley, the differences in percentage of germination from inoculated seed and control seed being so slight as to be negligible. *Cladosporium gramineum* also had very little if any effect on the seedlings, the percentage of germination from inoculated seed being only slightly smaller than from control seed.

To determine further how the *Helminthosporium gramineum* attacked the seed and seedlings, a large number of seeds and seedling plants grown from inoculated seed were dug and examined a few days after germination. It was found that many of the seeds had been attacked by the fungus so rapidly that they had not had an opportunity to germinate. Many others had germinated, apparently became infected immediately, and were killed before they were an inch high. Plants which survived were severely affected, as shown by the brown discoloration at the base of the culms, a condition not noticed in any of the controls. This discoloration usually occurred in the basal leaf sheath. When the plants had grown for several weeks, it was also very noticeable in the root crown. The discoloration was not as marked in barley grown from inoculated seed as the discoloration in wheat and was entirely absent in oats.

Numerous seeds and seedlings inoculated with *Fusarium culmorum* were also examined. Many seeds were found to have been killed before the process of germination had proceeded sufficiently far for any roots to form and before the plumule emerged from the ground. Eight days after

planting, the whole seed often was permeated by the fungus, the contents of the seed coats having a pink coloration. The plants which survived were discolored at the base in a manner similar to those of plants from seed inoculated with *Helminthosporium gramineum*. Where discolorations occurred, it was the first leaf sheath which was affected, while the central stem or culm was normal in appearance and color. The vigor of the plants from inoculated seed was markedly reduced, and they were shorter than the normal plants during the six weeks in which they were grown. This was true also of wheat and barley grown from seed inoculated with this fungus.

COMPARATIVE ROOT DEVELOPMENT OF WHEAT PLANTS GROWN FROM SEED INOCULATED WITH HELMINTHOSPORIUM GRAMINEUM AND FROM CLEAN SEED

To determine the comparative development of the root systems of surviving plants from seed inoculated with *Helminthosporium gramineum* and from clean seed, two pots of wheat containing five plants each, one grown from inoculated and the other from clean seed, were removed to the laboratory and the soil carefully washed away from the root systems. The roots were spread out by floating them in water and then drawing off the water. The difference in development of the root systems of the two sets of plants was very marked. The roots of plants from inoculated seed were discolored near the root crown. They were also much shorter and much less vigorous than roots of plants from clean seed; this is strikingly shown in Plate LXIII. Numerous other plants were examined, and it was found that in practically every case where inoculated seed had produced plants which survived, the root systems were less vigorous than in plants grown from clean seed.

SOIL INFECTION WITH HELMINTHOSPORIUM

To determine whether or not soil in which seed inoculated with *Helminthosporium gramineum* had been planted would remain sufficiently infected for any length of time to injure later plantings, inoculated seed was planted in pots in the greenhouse at Washington, D. C., on November 21, 1911, and the resulting plants were grown for five weeks and then cut off. Control pots were similarly planted with clean seed and the plants removed after five weeks. These pots were again sown on January 13, 1912, with wheat which had been previously sterilized in a 2.5 to 1,000 formalin solution. Of 150 seeds planted in the soil in which wheat plants had been grown from seed inoculated with *H. gramineum*, 104, or 69.3 per cent, germinated and produced plants, while of 90 seeds planted in control pots 76, or 84.4 per cent, germinated. This indicates that the soil remained infected during the two months in which the experiment was in progress. How long soil remains infected in this way is one of the important problems in plant pathology.

FIELD EXPERIMENTS WITH SEED INOCULATED WITH IMPERFECT FUNGI

In order to test whether the imperfect fungi which were found pathogenic in the greenhouse on seeds and seedlings would act similarly under field conditions, field experiments were undertaken at University Farm, St. Paul, Minn., in the spring of 1912.[1] The two strains of *Helminthosporium gramineum* and the one strain of *Fusarium culmorum* which had been found pathogenic in the greenhouse were tested in connection with wheat, barley, and oat seed. The same varieties of grains which were used in the experiments at Washington, D. C., were used in the field experiments. The seed was treated in a formalin solution of 3 parts of 40 per cent formaldehyde to 1,000 parts of water for one hour and afterwards was inoculated exactly as in the greenhouse work already described. Immediately after inoculation, the seed was planted in the field in rows 1 rod in length and 10 inches apart, with controls every alternate two rows. The seeds were counted. After the grain had sprouted and the plants were from 3 to 6 inches high, careful counts were made to determine the percentage of germination and observation made of the vigor of the plants during the first few weeks of growth. The results are given in Table III.

TABLE III.—*Results of inoculating seed of wheat, barley, and oats with imperfect fungi isolated from grain plants, and of planting them in the field at University Farm, St. Paul, Minn.*

Test No.	Species.	Origin.	Inoculated on—	Date of planting.	Inoculated seed.			Control seed.		
					Number planted.	Germinated.		Number planted.	Germinated.	
						Number.	Per centage.		Number.	Per centage.
				1912.						
1	Helminthosporium gramineum.	Wheat culm...	Wheat..	Apr. 27	160	125	78.1	159	155	97.5
2	.....do.....	.....do.....	Barley..	...do.....	160	115	71.9	160	130	81.2
3	.....do.....	.....do.....	Oats....	...do.....	160	145	90.6	160	142	88.7
4	.....do.....	Barley leaf....	Wheat..	...do.....	160	89	55.6	160	122	76.2
5	.....do.....	.....do.....	Barley..	...do.....	160	109	61.8	160	131	81.9
6	.....do.....	.....do.....	Oats....	...do.....	160	121	75.6	160	142	88.7
7	Fusarium culmorum.	Oat seedling..	Wheat..	...do.....	160	98	61.2	160	130	81.2
8	.....do.....	.....do.....	Barley..	...do.....	160	114	71.2	160	130	81.2
9	.....do.....	.....do.....	Oats....	...do.....	160	96	60.0	160	140	87.5

The results given in Table III substantiate the results of the experiments in the greenhouse. *Helminthosporium gramineum* from wheat when applied to the seed reduced the percentage of germination of both wheat and barley, but not to the same extent as in the greenhouse tests. Oats were not appreciably affected. The material used for inoculation

[1] In these experiments the writer was assisted by Messrs. Alden A. Potter and John H. Parker.

was not in a profusely sporulating stage and therefore not in as active a condition as the material which was used in the inoculations in the greenhouse. The seed which was inoculated also was still slightly damp after the treatment in the formalin solution and this trace of formalin might have reduced the effectiveness of the spores to some extent. The strain of *H. gramineum* from barley was more virulent than the strain from wheat, the percentage of germination being less where this strain was used for inoculation than where the strain from wheat was used. After inoculating with this strain, even the germination of the oats was considerably affected. The material used for inoculation, however, was in better condition than the material of the strain from wheat, as the fungus was sporulating abundantly when used. The plants of both wheat and oats which survived were less vigorous than the plants from clean seed, being slightly smaller than the plants in the control rows.

Fusarium culmorum also was virulent, particularly on oats, and its effect on wheat and barley was marked. The wheat plants which survived after inoculation with this fungus were smaller than those in the control rows, the difference being measurable. Several of the plants were dying when counted. In the case of barley the difference in the plants from inoculated seed and control seed was not marked, while in the case of oats many plants from the inoculated seed were very weak when counted, the difference in vigor between them and plants from clean seed being very noticeable. There was a sufficient difference in stand between rows from inoculated and from clean seed in the case of wheat, oats, and barley to be noticeable even without counting the plants.

That the reduction in germination and injury to seedlings was less marked in the field experiments than in the greenhouse experiments may be due to several causes. The temperatures in the field were considerably lower than under greenhouse conditions, and the fungi may have been less active for that reason. Again, the grain which had been treated with a formalin solution was not absolutely dry when inoculated and the trace of formalin present may have reduced the vitality of the spores. One other fact, however, which may have had a marked influence is that in the field the fungi used for inoculation would have to compete with other fungi and bacteria in the soil and many of the spores may have been injured before they could germinate and infect the grain. That such competition between fungi and bacteria in the soil may not be uncommon was indicated in a preliminary experiment in the greenhouse where wheat inoculated with *Helminthosporium gramineum* was planted in sterilized and unsterilized soil. It was found that the wheat planted in the sterilized soil was more severely injured by the fungus than the wheat planted in unsterilized soil, the percentage of germination being less in the sterilized soil than in the soil not sterilized. In a second experiment of this

nature the results were not as marked as in the first, although there was a difference in germination of 3.8 per cent between inoculated wheat planted in sterilized soil and inoculated wheat planted in soil which had not been sterilized.

A SYNOPSIS OF WORK RELATIVE TO HELMINTHOSPORIUMS AND FUSARIUMS ON CEREALS

The most comprehensive study of Helminthosporiums on grains is that of Ravn (20)[1] who isolated three species from barley and oats and by cultural and inoculation experiments, as well as a study of the morphology, definitely established their identity. Eidam (12) was the first to undertake inoculation experiments with species of Helminthosporiums. He inoculated barley with a strain of Helminthosporium secured from oats, but without positive results. Ritzema Bos (21) describes some of the diseases of barley in Holland and ascribes them to *H. gramineum*. Frank (13) describes a disease of barley which appears on the lower leaves of young plants and spreads gradually upward and believes it to be due to an infection of *H. gramineum*. Ritzema Bos (22) describes a disease on oats slightly different from a leaf spot in barley and believes it to be caused by *H. gramineum*. Pammel (18) describes a characteristic barley disease appearing in the United States and believes *H. gramineum* to be the causal organism. Many other investigators, both in Europe and this country, have studied the Helminthosporiums on grains with more or less definite results, and the literature on the subject is extensive. Practically all these studies, however, have been based on examinations of diseased plants and, with the exception of the work of Eidam, already quoted, have not been based on cultural and inoculation work. Hecke (14) secured a pure culture of *H. gramineum* from barley plants. He inoculated seedling barley plants both with mycelium and sclerotia and secured positive results in the formation of brown spots on the leaves. Ravn (20) cleared up the question of identity of three species of the Helminthosporiums attacking barley and oats. In extensive cultural and inoculation studies he obtained pure cultures. One of these he secured from stunted barley plants and established that it was the cause of deep-seated infection in the tissues of leaf, stem, and roots, while another species affected only the leaves, but was not systemic. The first he attributes to *H. gramineum*, the second to *H. teres* Sac. A similar disease on oats is attributed to *H. avenae* Br. and Cav. These three fungi were studied in pure cultures on beer wort and other culture media and found to differ in cultural characteristics, *H. gramineum*, after 14 to 20 days' growth on beer wort, producing a snow-white, uniformly smooth mycelium; *H. teres*, a much less abundant mycelium,

[1] Bibliographic citations in parentheses refer to "Literature cited," pp. 487–489.

which gathers more or less in masses; and *H. avenae*, a mycelium more nearly resembling *H. gramineum*, but less smooth and with more of a tendency to mass together. The developmental history and morphology of the mycelium and conidia in culture was very similar for the three species, but when the conidia were measured in large numbers those of *H. teres* were slightly longer than those of *H. gramineum* and those of *H. avenae* slightly larger than those of *H. teres*.

In a series of inoculation experiments *Helminthosporium teres* from barley transferred to barley, but not to oats, rye, or wheat; *H. gramineum* to barley, but not to oats; and *H. avenae* to oats, very slightly to barley, and not to rye.

Until Ravn made these intensive studies of the three Helminthosporiums they had been confused in the literature as to identity. The strain of *H. gramineum* discussed in this paper corresponds in cultural and morphological characteristics to the descriptions by Ravn.

Pammel, King, and Bakke (19) report a number of species of Helminthosporium on cereals in Iowa, among them *H. gramineum*. They cite inoculation tests to show that infection occurred when barley seedlings were inoculated with spores of this fungus and when the soil in which seedlings grew was inoculated. Beckwith (5) reports the isolation of undetermined species of Helminthosporium from old wheat soils, roots, and stems of wheat in North Dakota, but no inoculation experiments are mentioned. A comprehensive bibliography of the literature on Helminthosporiums up to 1900 is given by Ravn (20).

The literature relating to Fusariums on grains is also very extensive. Chester (10) reports that *F. culmorum* is the cause of the disease known as scab of wheat and shows that many shrunken wheat kernels contain a fungous mycelium. Detmers (11) shows that the disease known as wheat scab in Europe and caused by *F. culmorum* has become prevalent in America. Selby (30) ascribes wheat scab in Ohio to the fungus *F. roseum* Link. and believes the conidial form of *Gibberella saubinetti* to be its conidial stage. Some field inoculations with Fusarium attempted by him were unsuccessful.

The first investigator to show with any degree of certainty that Fusarium infection can be carried with the seed is Rostrup (25, 26, 27, 28). Ritzema Bos (24), Westerdijk (35), Volkart (34), Appel (1, 2), and Selby and Manns (31) came to similar conclusions. Sorauer (32, 33) was the first to prove that infection could be carried with the seed. He maintains, however, that infection in this manner is of small consequence as compared with infection through the soil.

Selby and Manns (31), in their studies on the form Gibberella, conclude that this fungus attacks rye, oats, barley, and spelt. Inoculations on wheat with pure cultures of *Gibberella saubinetti* (Mont.) Sacc. from perithecia on wheat reduced germination to the extent of 17.1 and 32.4 per cent, respectively. Similar results on both wheat and oats were obtained by them with *Fusarium roseum* from wheat and clover.

Appel (3) believed that infection with *Fusarium nivale* Ces. is due principally to soil infection, while Hiltner and Ihssen (15) believe that seed infection is of more importance.

Muth (17) carried on pure culture inoculation experiments on rye with *Fusarium roseum*. In these, 55 per cent of the inoculated seed sprouted while only 63 per cent of the controls sprouted. A large number of plants from inoculated seed, however, showed the results of infection through a yellowish or yellowish brown discoloration of the roots.

Beckwith (4) reports numerous isolations of Fusarium species and other imperfect fungi from stems and roots of wheat grown on soil continuously cropped to wheat and from the soil itself.

Mortensen (16) demonstrated that rye seed heavily infected with *Fusarium nivale* Ces. produced diseased plants. He states that not only *F. nivale* but other Fusariums produce root diseases in cereal plants.

Bolley (6), from extensive field studies on wheat from land continuously cropped to wheat, has come to the conclusion that "through the practice of continuous wheating, soils in many cases have become infected with from one to three or four definite parasitic fungi which attack in the same manner as the flax-sick fungi attack and destroy the flax crop on flax lands and, therefore, such wheat lands may be said to be 'wheat sick.'" These views are further elaborated by him from extensive field studies and observations (7, 8). Bolley (9) also reports on the isolation of a considerable number of imperfect fungi from the nodes and internodes of wheat plants grown on experimental plats at the North Dakota Agricultural Experiment Station. Among them undetermined species of Helminthosporium and Fusarium occurred in abundance. No inoculation experiments are reported.

Schaffnit (29) in a comprehensive work on "Schneeschimmel" gives a discussion of the fungus *Fusarium nivale* with relation to its occurrence, morphology, cultural characteristics, physiology, and preventive measures. He shows that this disease is due both to soil infection and seed infection, the former being more common. Incidental to his work on *F. nivale* Schaffnit (29) performed some inoculation experiments with *F. rubiginosum* Appel and Woll. on etiolated rye seedlings in damp atmosphere with positive results. The number of inoculations is not stated. *F. rubiginosum* has recently been demonstrated by Dr. H. W. Wollenweber to be identical with *F. culmorum*. A comprehensive bibliography of literature dealing with Fusariums on cereals is given by Mortensen (16).

CONCLUSIONS

The experiments described in this paper and the literature cited show that some of the imperfect fungi occurring on small grains and inducing leaf spots or systemic infections are pathogenic when, under favorable conditions, they come in contact with seeds and seedlings,

while other forms apparently are nonparasitic. *Helminthosporium gramineum* and *Fusarium culmorum* were found to be parasitic, while *Cladosporium gramineum* and an undetermined species of Altenaria were not parasitic under the conditions here described. That only certain species are pathogenic is to be expected. Their identity as well as that of the large number of forms apparently saprophytic on cereals is more or less confused in the literature but should be determined, and the extent to which these fungi affect cereals should be ascertanied by laboratory and greenhouse studies. These need to be reinforced by pure culture inoculations of seeds, seedlings, plants in various stages of growth, and soil under field conditions before the exact relation of such fungi to cereal cropping can be definitely established.

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PLATE LXII

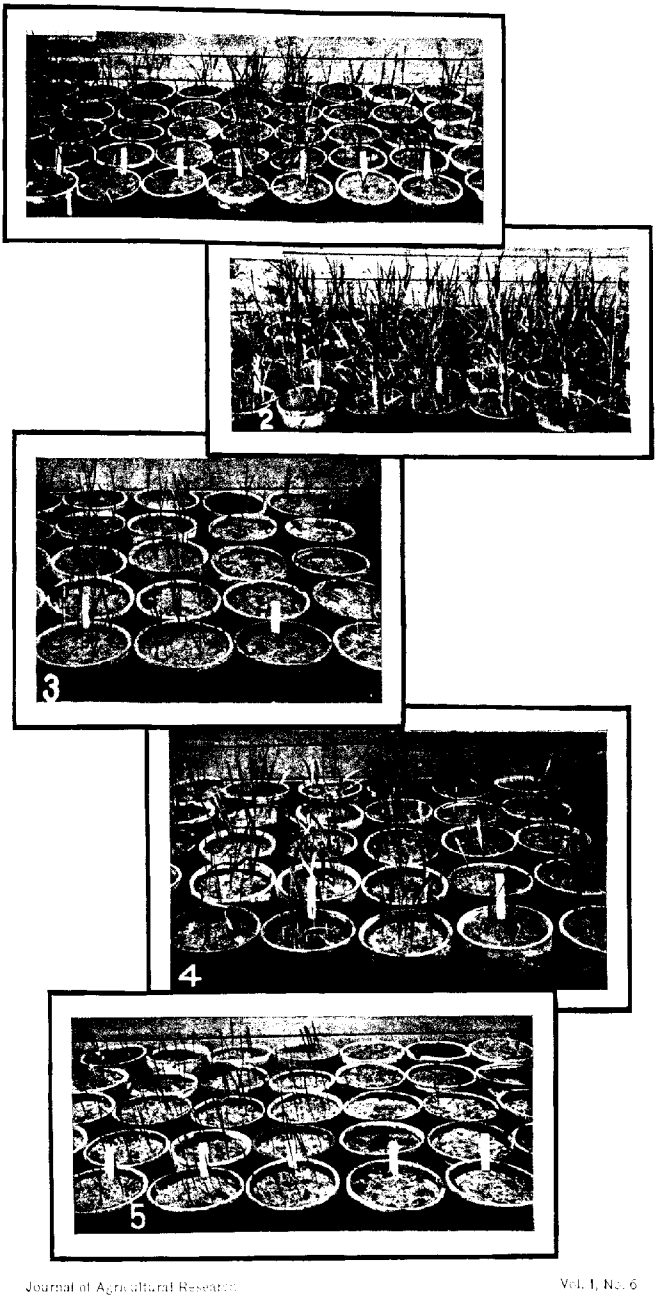
Fig. 1.—Wheat seedlings from seed inoculated with spores of *Helminthosporium gramineum* and from seed externally sterilized; photographed three weeks after planting. The three rows of pots on the left were sown with seed inoculated with spores of *H. gramineum* from barley, the two rows in the center with sterilized seed, and the three rows on the right with spores of *H. gramineum* from wheat.

Fig. 2.—Barley seedlings from seed inoculated with *Helminthosporium gramineum* and from sterilized seed; photographed three weeks after planting. The three rows of pots on the left from seed inoculated with spores of *H. gramineum* from barley, the next three rows from seed externally sterilized, and the row on the right from seed inoculated with *H. gramineum* from wheat.

Fig. 3.—Wheat seedlings from seed inoculated with spores of *Fusarium culmorum* from oat seedlings (two rows of pots on right) and from seed externally sterilized (two rows of pots on left). Photographed two weeks after planting.

Fig. 4.—Barley seedlings from seed inoculated with spores of *Fusarium culmorum* from oat seedlings (two rows of pots on right) and from seed externally sterilized (three rows on left). Photographed two weeks after planting.

Fig. 5.—Oat seedlings from seed inoculated with spores of *Fusarium culmorum* from oat seedlings (two rows on right) and from seed externally sterilized (three rows on left). Photographed two weeks after planting.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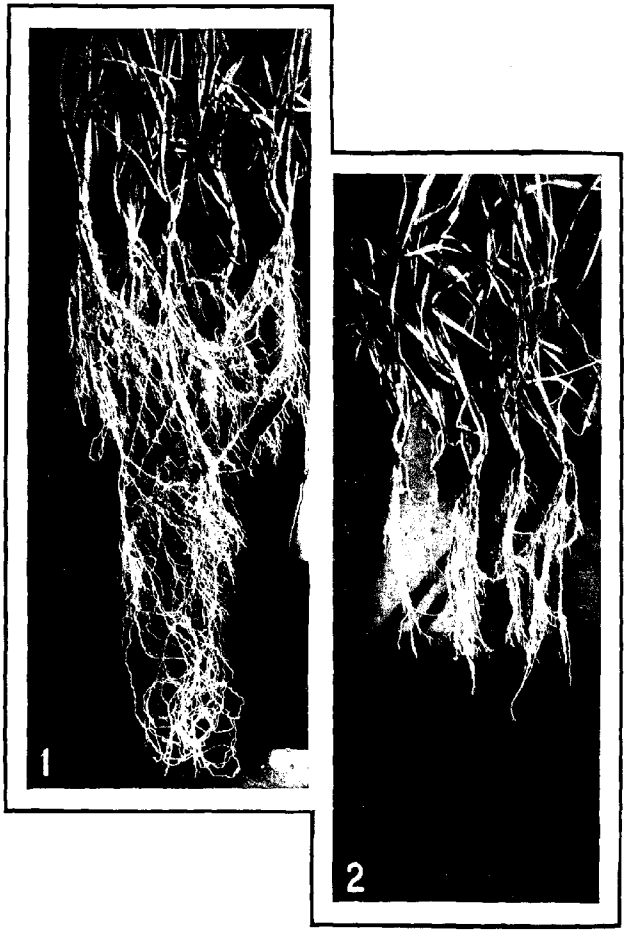
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PLATE LXIII

Root systems of wheat seedlings grown in 6-inch pots from seed externally sterilized (left) and from seed inoculated with *Helminthosporium gramineum* from wheat (right). Photographed six weeks after planting.

THE ORIGIN OF SOME OF THE STREPTOCOCCI FOUND IN MILK

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INTRODUCTION

In the higher plants and animals we are accustomed to associating species with a more or less definite habitat. Certain animals are found only in certain localities. One species of trees may be found only on a particular type of soil. A still narrower limit of distribution is found in some of the parasitic fungi which grow only on closely related host plants. Zoologists or botanists find the types on which they base their descriptions in the natural habitat of the organism. This relation has not always existed in the published descriptions of bacteria. The association of a natural group with a particular habitat has been more or less incidental, except perhaps with the pathogenic bacteria, and even with some of these it is not impossible that the pathological conditions under which they are found may not be the true habitat of the species. The colon group, while it is frequently found in water and milk, has its natural habitat in the intestinal tract of warm-blooded animals. Winslow found that certain chromogenic cocci were associated with the skin of animals.[1] Some of the English bacteriologists have pointed out that the streptococci from horse manure, for instance, have a set of physiological reactions which differentiates them from those from saliva or pathological conditions.[2] It is only through a knowledge of the habitat and the study of sufficient cultures to establish a type that true bacterial species can be determined. If we were to write a description of the German people we would go to Germany, not to an American city where German immigrants live.

Countless descriptions have been written of bacteria isolated from milk until we have come to consider certain types as peculiar to this medium. The bacteria found in milk, however, are a heterogeneous collection, and the true types of milk bacteria are to be sought in the sources from which milk is contaminated. Esten has suggested that the streptococci or lactic-acid bacteria of milk come originally from the mouth of the cow.[3] The feces of the animal must, unfortunately,

[1] Winslow, C. E. A., and Winslow, Anne R. Systematic relationships of the Coccaceæ. ed. 1, 300 p., illus. New York, 1908.

[2] Andrewes, F. W. Report on the micro-organisms present in sewer air and in the air of drains. 36th Ann. Rpt. Local Govt. Bd. [Gt. Brit.], 1906–07, Suppl. Rpt. Med. Off., p. 183–204. 1908.

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be considered as a possible source of bacteria in milk, among which would undoubtedly be found members of the lactic group. Kinyoun and Dieter believe that the presence in milk of cocci which form chains in lactose bile at 37° C. is presumptive evidence that the milk is contaminated with feces.[1] It is the more common practice, however, to consider this type as the indication of the presence in the herd producing the milk of one or more cows with infected udders.

The mouth is known to contain streptococci, and the habit of cows of licking their flanks and udders provides a more or less direct connection between the mouth and the milk pail. Each of these sources may be considered as the normal habitat of bacteria. Under these conditions they persist for indefinite generations, adapting themselves to their environment until it is reasonable to suppose the characters acquired become sufficiently fixed to have at least varietal significance.

The study of streptococci originating within such circumscribed limits is of interest in addition to its taxonomic importance, in the light it may cast on the origin of some of the bacteria in milk and the significance from the hygienic standpoint of the presence of certain types.

In this paper are recorded the results of a study of streptococci representing three of the possible sources from which this group may find its way into milk. The morphology of this collection was studied with the hope that this would give some basis for a division into varieties. The ability of these cultures to utilize a number of carbohydrates and alcohols was determined. On the basis of these fermentations several groups are established, each of which is made up of a large number of identical cultures constituting the type about which are grouped similar cultures, but which varied from it in one or two reactions. The probable relation of one of these groups to well-known species is pointed out.

THE CULTURES STUDIED

A collection of streptococci were obtained from milk, from bovine feces, from the mouths of cows, and from the udders of cows. With the exception of those from milk an effort was made to make the cultures as representative as possible. The procedure of isolating the milk cultures followed that usually employed in the laboratories of boards of health. Small portions of the milk were added to lactose-bile tubes which were incubated at 37° C. Tubes showing streptococci in distinct chains on microscopical examination were plated on lactose agar and the chain-forming cocci subcultured. In this way 42 cultures were isolated from 25 samples of milk and cream collected at Washington or at the creamery at Troy, Pa. No two samples came from the same farm. A few cultures were obtained through the courtesy of Dr. Kinyoun and Mr. Dieter from lactose-bile tubes in the laboratory of the health department of the District of Columbia. These cultures, therefore, did not

[1] Kinyoun, J. J., and Dieter, L. V. A bacteriological study of the milk supply of Washington, D. C. Jour. Amer. Pub. Health Assoc., v. 2, no. 4, p. 262–274. 1912.

represent the normal streptococci of milk but rather those which would usually be distinguished as indicating contamination from infected udders or fecal sources.

Fifty-one cultures were isolated from 19 samples of milk obtained by milking directly into sterile test tubes. The cows from which these samples were obtained represented all gradations of infected udder from occasional evidence of garget to acute mammitis. Part of these were in the Dairy Division herd at Beltsville, Md., and the remainder in the herd on the Naval Academy farm at Annapolis, Md. One hundred and fourteen cultures came from 56 samples of cow manure obtained, with the exception of a few from Troy, Pa., at the Dairy Division farm and at the dairy of the Government Hospital for the Insane at Washington. Thirty-nine cultures were made from the mouths of animals at the Dairy Division farms. With the exception of one culture obtained from the mouth of a mule, all of these cultures were of bovine origin. In Table II the origin of the culture is indicated by M for milk, U for udder, F for feces, and B for mouth. The sample from which the culture was secured is indicated by a number following the letter. For instance, "F15" represents sample of feces No. 15. This will enable the reader to determine the origin of each culture and the number of cultures from each sample.

MORPHOLOGY OF THE CULTURES

While it is generally recognized that there is little morphological basis for subdivisions of the streptococci, reference is frequently made to certain types of cells. Stowell, Hilliard, and Schlesinger,[1] in selecting streptococci from milk for comparison with those isolated from the human throat, rejected diplococci and the oval-chained form which they designate as the *Streptococcus lacticus* of Kruse or the *Bacillus lactis acidi* group, respectively. In selecting our cultures no attention was paid to morphology beyond determining that it was a coccus apparently dividing in one plane, with the exception of those from milk, which were not accepted if they did not form chains of at least 8 or 10 cells. The morphology of nearly all cultures was determined by examination of specimens stained with gentian violet. Camera-lucida drawings were made using a Leitz 3 mm. objective and No. 18 ocular, a combination which gave a magnification of 2,400 diameters at the ocular, or 4,800 diameters on the drawing board. Sufficient light to give a clear image was obtained by using a special arc light with a copper-sulphate ray filter.

Preliminary studies showed that the medium on which the culture was grown had an appreciable influence on both the size and the form of the cell. This is shown in figure 1, which is reproduced from camera-lucida drawings of typical cultures grown on various media. Milk gave quite

[1] Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J., A statistical study of the streptococci from milk and from the human throat. Jour. Infect. Diseases, v. 12, no. 2, p. 144-164. 1913.

uniformly smaller cells and less tendency to chain formation than broth or agar. The cells at *a* are from culture *lo* on milk, *b* on broth, and *c* on agar, all incubated 48 hours at 37° C. The difference between the distinctly rod-shaped cell found on agar and the small round cell obtained from milk is marked. That differences in size of cells are not due entirely to differences in the medium is shown by the chain at *h*. This combination of small and large cells in a single chain is not unusual in broth, a medium in which there is a marked tendency to form enlarged and abnormal cells. In some cultures the transition from normal cells to those of monstrous size and form was so rapid that it was difficult to obtain preparations showing what could be considered normal cells. The most satisfactory preparations were obtained in incubating broth cultures until a distinct cloudiness was obtained, centrifuging the culture, siphoning off the broth, and washing the sediment with sterile water. After centrifuging again the water was siphoned off, and a preparation made from the sediment. This gave a clear field suitable for examination under a high-power microscope.

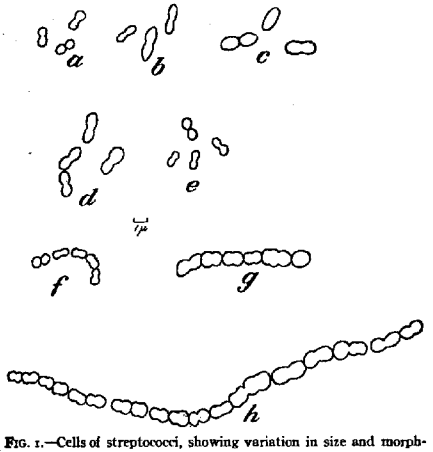


FIG. 1.—Cells of streptococci, showing variation in size and morphology. *a*, culture *lo* on milk; *b*, culture *lo* on broth; *c*, culture *lo* on agar; *d*, culture *li* on lactose bile; *e*, culture *li* on broth; *f*, culture *gm* on milk; *g* and *h*, culture *gm* on broth.

Various types of cells which were found in this collection are shown in figure 2. It will be observed that much of the variation in these types is in size only or in chain formation. The slender-pointed cells shown at *F* were peculiar to the cultures obtained from the mouth of animals, but the cultures from this source were not confined to this type. In Table II the letter under the heading "Morphology" refers to figure 2, although it is obvious that in many cases the assignment to a particular type can be only an approximation. The variation of the morphology is so great and so easily affected by the environment that it was not considered in the final arrangement of groups. It should be stated, however, that among the udder cultures the tendency to chain formation was much more marked and more constant than among all other cultures.

METHODS OF DIFFERENTIATION

When morphological distinctions are lacking, we are forced to use the physiology of the organism as a basis of classification. No single system

of characters can be adopted for all classes of bacteria. The significant characters will be found for each group only by a study of its normal activities and the utilization of those functions which show the nature, limitations, and relationship of the group. The striking characteristic of the streptococci is their ability to form acids from carbohydrates and related substances, and this peculiarity has been very generally utilized for purposes of classification. The voluminous literature bearing pro and con on the constancy and the value of these tests has been reviewed fully in various papers and need not be taken up here. It may be safely asserted, however, that the fermentative ability is as constant and as significant for purposes of classification as the characters adopted by those who reject the fermentation tests as too variable. For instance, Davis, who rejects the sugar fermentations as untrustworthy, divides the

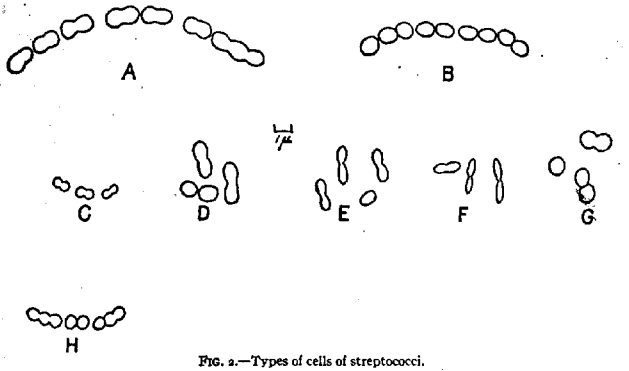


FIG. 2.—Types of cells of streptococci.

streptococci into five groups on the basis of hemolysis, green colonies on blood agar, capsule, solubility in bile, inulin fermentation, experimental arthritis, and experimental endocarditis.[1]

For purposes of classification, we have used the liquefaction of gelatin and the fermentation of dextrose, saccharose, lactose, raffinose, starch, inulin, mannite, and glycerin. Adonite and dulcite were tested, but as they were fermented by only one or two of these cultures they were of no value. The liquefaction of gelatin was determined by inoculating the surface of the gelatin tube with a few drops of a broth culture and measuring the liquefaction after 30 days at 20° C.

The fermentation of the test substances was determined in a medium made as follows:

	Per cent.
Beef extract	0.4
Peptone	1.0
Dibasic potassium phosphate	.5
Test substance	2.0

[1] Davis, D. J. Interrelations in the streptococcus group with special reference to anaphylactic reactions. Jour. Infect. Diseases, v. 12, no. 3, p. 386. 1913.

The cultures were incubated for seven days at 30° and titrated cold against twentieth-normal sodium hydrate with phenolphthalein as an indicator. From the results so obtained is subtracted the titration of a blank, and the result is expressed as the percentage of normal acid. Some objection may be raised against the use of 30° C. as an incubation temperature rather than the more common one of 37°. The lower temperature was adopted because practically all streptococci will grow at this temperature, while a few grow at 37° slowly or not at all.

The fermentation produced by the streptococci is in almost all cases so marked that there is very rarely any question about the presence or

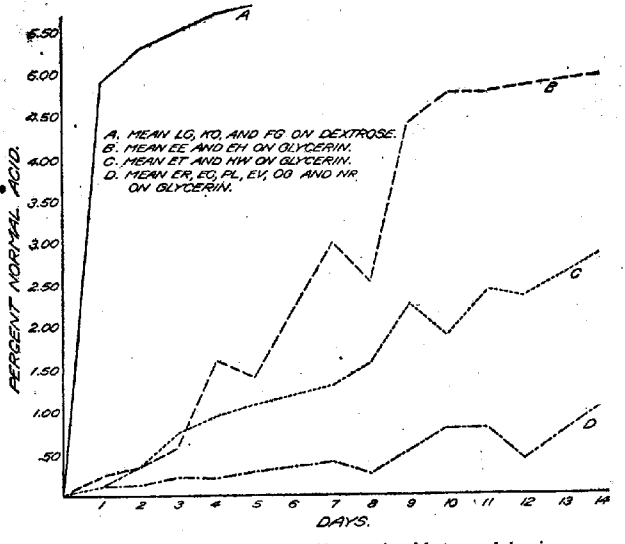


FIG. 3.—Curve showing the typical rate of fermentation of dextrose and glycerin.

absence of the fermentation. Of all the substances we have used glycerin forms an exception to this rule. The fermentation proceeds slowly and in seven days may be slightly above or slightly below 1 per cent normal acid, the point selected as marking the line between fermentation and no fermentation. This is illustrated by Table I, which shows the progressive rate of fermentation by typical cultures. Three cultures fermenting dextrose are included to show the usual course of the fermentation in the more easily fermented sugars. Each titration was made from a separate tube. A study of this table shows that the 12 cultures may be divided into three quite distinct types on the basis of the rate of fermentation of glycerin. This is shown more clearly in figure 3, in which the average titrations for each of the three types are plotted. Two of these cultures fermented the glycerin with comparative

rapidity and after three days there was no question that the cultures were able to utilize glycerin. Those represented by the curve *D*, on the other hand, produce only a very slight increase in acidity, which even at the end of 14 days is only slightly above 1 per cent normal.

Between these two is a third group in which there is a slow but distinct increase in acidity. At seven days the acidity is above 1 per cent normal. While an error may be introduced in some cases by drawing the line between fermentation and no fermentation of glycerin at 1 per cent normal, it is believed that in these results this error will be slight. These results illustrate the value of the exact results obtained by titration which we have always used in preference to the simpler and more rapid way of determining the change of reaction with litmus in solution in the broth or with litmus papers.

We also consider it a decided advantage to allow sufficient time for the completion of the fermentation, thus securing an end point rather than some intermediate and varying determination. Seven days are not sufficient for the completion of the glycerin fermentation, but it is undoubtedly ample for other test substances which we have used.

TABLE I.—*Progressive fermentation of dextrose and glycerin.*

DEXTROSE.

Culture No.	Percentage of normal acid produced in—											
	1 day.	2 days.	3 days.	4 days.	5 days.	7 days.	8 days.	9 days.	10 days.	11 days.	12 days.	14 days.
lg	4.6	5.2	5.2	5.6	5.5	.....	.....	.....	.....	.....	.....	.....
ko	4.6	5.0	5.2	5.5	5.5	.....	.....	.....	.....	.....	.....	.....
ig	5.2	5.8	6.2	6.1	6.5	.....	.....	.....	.....	.....	.....	.....

GLYCERIN.

ec	0.15	0.19	0.31	0.34	0.44	0.55	0.58	0.91	0.53	0.96	0.78	1.21
ee	.25	.23	.80	1.67	1.31	3.65	2.73	4.96	4.85	4.96	.....	5.43
eh	.22	.46	.35	1.57	1.54	2.40	2.38	3.96	5.06	4.61	3.74	4.68
er	.15	.14	.37	.37	.96	.86	.28	.63	1.83	.92	.33	1.13
et	.05	.59	.82	.95	1.09	1.30	1.49	2.16	1.73	2.18	2.30	2.63
ev	.15	.09	.34	.17	.00	.40	.45	.66	1.03	.96	.70	1.18
hw	.20	.11	.67	.95	1.07	1.35	1.67	1.38	2.08	2.71	2.45	3.15
mr	.15	.09	.14	.00	.00	.25	.02	.31	.43	.76	.21	.....
ny	.20	.29	.04	.00	.05	.03	.00	.01	.00	.21	.00	.....
og	.21	.09	.02	.07	.09	.05	.02	.11	.48	.46	.03	.....
om	.15	.00	.09	.11	.09	.06	.00	.22	.13	.19	.00	.00
pl	.05	.09	.22	.32	.24	.36	.31	.71	.53	.76	.66	.78

THE CORRELATION OF PHYSIOLOGICAL CHARACTERS

The complete results of the tests made on this collection of cultures are presented in Table II. The reduction of neutral red and the curdling of milk are given in the table, but are not used in the correlations. Adonite and dulcite are necessarily excluded, since these cultures almost without exception fail to ferment these two substances.

TABLE II.—*Physiological characters of all cultures.*

Culture No.	Origin.	Morphology.	Chains. Milk.	Chains. Broth.	Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid. Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
ec	M1	G	....	—	0	—	+	4.1	0	0.1	4.3	0	1.3	0	0	0.6	0.1
ed	U1	G	....	—	30	+	+	1.7	.1	4.0	4.0	0	.4	0	2.4	.8	0
ee	U1	G	....	—	28	+	+	2.5	0	4.1	4.2	.1	.2	.2	2.9	.8	.1
eh	U1	G	....	—	27	+	+	2.6	0	3.6	3.8	.2	.2	0	1.8	.9	0
ej	U2	B	....	+	0	—	+	2.7	0	2.7	4.6	0	0	.1	0	.1	.1
em	M2	G	....	—	0	—	+	4.4	0	0	4.5	.1	.2	.1	2.3	.6	0
eo	M3	GE	....	—	50	—	+	4.3	.1	4.7	5.1	.2	.4	.5	4.4	1.7	.2
eq	M4	GE	....	—	0	—	+	4.1	.1	4.8	4.8	0	.2	.1	4.0	2.1	.1
er	M5	G	....	—	0	—	....	4.6	0	.2	5.4	0	.2	0	.1	.7	0
et	M6	G	....	—	0	—	+	6.5	0	4.1	5.1	.1	.1	.2	3.9	.9	0
eu	M7	G	....	—	0	—	+	6.6	0	.4	5.0	0	0	0	2.4	.4	0
ev	M7	G	....	—	0	—	+	3.0	0	.3	5.0	0	0	.1	2.1	.3	0
ew	U1	G	....	—	0	—	—	4.9	0	3.9	3.6	0	0	0	1.5	.7	0
ez	U1	G	....	—	19	—	—	4.7	0	3.0	3.6	.1	1.1	0	.9	.6	0
fa	U1	G	....	—	55	—	+	2.2	.2	3.4	3.2	.2	.3	.4	.8	.8	.2
fc	U2	D	....	—	0	—	—	1.8	0	0	.4	0	0	0	0	0	.2
fg	U2	EG	....	—	49	—	+	6.6	0	.4	5.6	0	.3	.2	6.2	2.0	.2
fi	M8	....	....	—	0	—	+	5.3	0	5.6	5.6	.2	.1	0	3.9	0	.1
fj	M9	C	....	—	0	—	+	5.6	0	5.3	4.9	.1	0	0	3.5	0	0
fk	M10	....	....	—	0	—	+	7.1	.2	.2	5.7	.3	0	.1	0	.5	.2
fl	M11	....	....	—	0	—	+	5.0	0	.4	5.2	.4	.2	.2	4.3	1.3	.1
fn	M12	....	....	—	....	—	—	5.1	0	5.5	5.3	0	.2	0	4.2	0	0
fo	M12	G	....	—	0	—	....	5.5	.1	5.6	4.5	0	0	0	4.4	.1	0
fp	M8	E	....	—	0	—	....	5.9	.1	5.8	4.7	0	.2	0	4.3	.4	0
fr	M13	G	....	—	0	—	+	5.3	0	4.8	5.5	.3	0	.1	4.3	1.2	.1
ft	M9	....	....	—	0	—	+	5.4	0	5.1	5.3	0	.1	.1	2.7	.2	.1
fu	M10	G	....	—	0	—	+	7.0	0	.1	5.2	0	0	0	.1	.5	0
fv	M10	....	....	—	0	—	+	6.6	0	.1	5.6	.1	.1	.1	0	.5	0
fw	M14	....	....	....	0	—	+	5.4	0	4.8	5.2	.2	.1	.1	4.1	1.2	.1
fx	M15	C	....	—	0	—	....	5.3	0	5.4	4.6	0	0	0	3.8	.1	0
fy	M15	....	....	....	0	—	....	5.3	0	5.5	5.2	.2	.1	0	4.3	1.1	0
ga	M16	....	....	....	0	—	....	6.0	0	0	5.6	.2	0	0	.1	.1	0
gb	M16	....	....	....	0	—	+	6.5	.1	.1	5.4	0	.1	.2	.1	.4	.1
gc	U3	H	....	+	0	—	—	4.4	0	3.6	4.0	0	.1	3.8	0	0	0
ge	U3	....	+	—	0	—	+	4.6	0	4.7	4.8	.3	.4	0	0	.1	1.9
gh	U4	....	+	—	0	—	+	4.2	0	0	4.9	.1	0	.1	0	.1	0
gi	U5	H	....	+	....	—	+	4.8	0	.1	4.3	0	.2	0	0	.1	0
gk	U5	B	+	+	0	—	+	4.5	.1	4.4	4.8	.1	0	.1	0	.3	0
gl	U5	....	....	....	....	—	+	4.6	0	0	4.3	0	0	0	0	0	0
gm	U5	A	+	+	0	—	+	4.4	.1	4.7	4.9	.2	0	0	0	0	0
go	U6	....	+	....	0	—	+	4.5	0	4.8	4.8	0	0	0	0	0	0
gp	U5	....	+	....	....	—	+	4.3	.3	0	4.2	0	.7	.2	0	.1	0
gt	f1.	....	—	....	0	—	+	4.7	.3	4.6	4.6	.1	.4	0	0	.3	0
gw	U6	....	+	....	0	—	+	4.5	0	4.6	5.0	.1	0	0	0	0	.1
gx	U6	....	+	....	0	—	+	4.5	.1	4.9	5.0	.1	0	.2	0	0	0
gz	U2	B	....	+	0	—	+	4.7	0	0	4.4	0	0	0	0	0	0
ha	M17	E	—	—	0	—	+	6.4	0	0	5.1	.1	0	0	2.3	.3	0
hb	M17	E	—	—	0	—	+	6.4	.2	0	5.1	.1	0	.1	3.8	.4	.1
hc	M17	....	—	—	0	—	+	5.5	.1	0	5.9	.1	.2	0	.2	.5	0
hd	M18	E	—	—	0	—	+	5.8	0	5.6	5.2	0	0	.1	3.9	.4	0
he	M18	E	—	—	....	—	+	6.1	0	5.5	5.6	.1	0	0	3.4	.4	.4
hg	M19	G	—	—	0	—	+	5.9	.2	.2	5.5	.1	.1	.1	1.2	.7	0
hh	M19	....	—	....	0	—	+	6.2	.1	0.1	5.5	.1	.2	.2	0	0.6	0
hj	M20	....	—	....	0	—	+	6.8	0	0	4.7	.1	.2	0	0	.4	0
hk	M21	....	—	....	....	—	+	4.3	.2	.2	5.5	.1	.1	0	0	.5	.1
hl	M21	....	—	....	0	—	+	7.1	.2	0	5.6	0	0	0	0	.5	.1
hm	M22	E	—	—	0	—	+	6.1	.4	0	5.3	.1	.1	.1	2.8	.3	0
hn	M22	....	—	—	0	—	+	5.6	.2	0	6.3	.1	.1	0	2.7	.4	0
ho	F2	....	—	—	0	—	+	5.7	0	6.0	5.5	5.3	0	.1	0	0	0
hq	F3	E	—	—	....	—	+	6.4	.2	5.5	5.3	0	0	0	.1	.1	0
hr	F3	....	—	....	....	—	+	4.7	....	....	5	....	....	....	....	0	.1
hs	F4	—	—	0	....	—	+	5.8	.3	5.8	5.7	5.3	0	0	0	.1	0
hu	M23	E	—	—	....	—	+	6.0	.1	5.5	5.2	.3	.3	0	4.6	0	0
hv	M23	E	—	—	0	—	+	6.2	.7	5.1	.7	0	0	0	5.0	1.5	.1
hw	M24	....	—	....	0	—	+	5.3	.3	4.8	5.1	.2	.2	0	4.3	.5	0
hx	M24	....	—	....	0	—	+	5.0	.1	4.7	5.2	.2	.2	.1	4.1	1.5	0
hy	F5	....	—	....	0	—	+	5.7	.2	5.9	5.5	5.3	4.7	0	0	0	.1
hz	F5	....	—	....	0	—	+	5.1	0	5.4	5.1	5.0	4.7	0	0	.1	0
ib	F6	....	—	....	0	—	+	5.8	.2	6.4	5.8	5.1	0	0	4.3	.2	0
ic	F6	....	—	....	0	—	+	5.7	0	6.7	5.6	5.2	0	0	4.3	.2	0
id	F7	....	—	....	0	—	+	5.7	0	5.8	5.6	5.4	0	0	0	0.1	0
ie	F7	....	—	....	0	—	+	5.7	0	6.0	5.4	5.3	0	0	0	0	0
if	F8	....	—	....	0	—	+	4.9	0	5.5	5.4	0	0	0	0	0	0.1
ig	F8	....	—	....	0	—	—	4.3	0	4.6	5.2	5.9	0	0.1	0	.1	0
ih	F9	G	....	—	0	—	....	6.1	.2	5.5	4.8	1.8	0	0	1.7	.2	0
ii	F9	....	....	....	0	—	—	7.7	.1	8.9	5.4	3.2	0.2	0	1.4	.3	0

TABLE II.—*Physiological characters of all cultures*—Continued.

Culture No.	Origin.	Morphology.	Chains. Milk.	Chains. Broth.	Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid. Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
					mm.												
ik	M25	G	—	—	0	—	+	5·2	0·2	4·3	4·7	3·2	0·2	2·9	4·0		
il	M25		—	—	0	—	+	4·6	·2	4·4	4·7	3·5	0·2	3·2	3·7	·2	·1
im	F10	E	—	—	0	—	+	6·5	0	6·1	5·0	4·8	4·6	0	·3	·1	0
in	F10		—	—	0	—	+	4·8	0·2	5·5	5·5	5·1	4·4	0	0	0	0
io	F11		—	—	0	—	+	5·8	0	5·8	5·1	5·0	·2	0	0	·5	0
ip	F12		—	—	0	—	+	5·4	·1	5·4	5·6	5·0	·1	0	0	·2	0
iq	F13	E	—	—	0	—	+	5·6	·2	7·6	5·7	4·5	·3	0	1·8	·2	0
ir	F13		—	—	0	—	+	4·8	·1	5·6	5·9	5·9	4·4	0	0	0	0
is	F13		—	—	0	—	+	5·5	·1	5·7	5·9	6·0	4·7	·1	0	0	·1
it	F13		—	—	0	—	+	5·5	0	5·3	4·9	4·8	6·2	0	0	·4	0
iu	F5	E	—	—	0	—	+	6·1	·2	6·2	5·4	·1	·2	0	5·3	·8	0
iv	F5		—	—	0	—	+	5·5	·1	6·2	5·8	5·1	4·5	0	4·1	·2	0
ix	F14		—	—	0	—	+	6·9	0	6·0	5·2	4·7	6·2	0	0	0	0
id	F15	D	—	—	0	—	+	4·3	·1	5·4	5·1	5·0	0	0	0	0	0
ie	F15		—	—	0	—	+	5·6	·2	6·3	5·5	5·3	0	0	0	0	0
if	F15	E	—	—	0	—	+	5·7	·1	5·8	5·5	5·0	4·4	0	0	0	0
ig	F16		—	—	0	—	+	4·5	·2	4·2	5·1	0	·1	0	0	·1	0
ih	U7		+	—	0	—	+	4·5	·1	4·6	4·9	0	·2	0	0	·1	0
ii	U7		+	—	0	—	+	4·7	0	4·4	4·6	0	0	·1	0	·1	0
ik	U2		—	—	0	—	+	4·8	·1	4·9	4·4	·1	0	·1	0	·1	·1
il	U5		—	—	0	—	+	3·4	·1	4·8	4·5	·3	·1	·2	0	·2	·1
im	U5		+	—	0	—	+	4·8	0	4·5	3·6	·2	·3	3·9	2·7	·2	0
in	U3	H	—	+	0	—	+	4·8	·1	3·8	0	·1	·2	0	3·6	0	0
io	U8		—	—	0	—	+	4·5	·1	3·8	4·0	0	0	0	3·4	·1	·1
ip	U8		—	—	0	—	+	6·4	·2	6·2	4·9	4·9	0	0	·3	0	0
iq	F17	D	—	—	0	—	+	5·5	·1	5·8	5·3	5·2	0	·1	0	·1	0
ir	F17		—	—	0	—	+	5·4	·2	5·9	5·3	5·1	4·8	0	·2	·1	0
is	F17	G	—	—	0	—	+	5·8	·1	4·9	5·2	4·9	3·9	6·4	0·1	·1	0
it	F18	E	—	—	0	—	+	4·9	0	5·1	4·9	5·5	·1	·2	0·1	0	·1
iu	F18		—	—	0	—	+	5·9	0	4·8	4·9	5·1	6·0	5·8	0	0	·1
iv	F19		—	—	0	—	+	3·5	0	5·3	5·4	6·0	·2	0	·3	·1	0
iw	F20		—	—	0	—	+	5·4	·2	5·8	5·1	5·1	·1	0	0	·2	0
ix	F20	E	—	—	0	—	+	5·8	0	5·8	5·5	5·2	0	0	0	·1	0
iy	F20		—	—	0	—	+	6·9	0	0	5·5	0	·2	·1	0·1	0	·1
iz	F21		—	—	0	—	+	5·5	·1	4·9	5·3	4·8	4·6	·1	0	·1	0
ka	F21		—	—	0	—	+	5·4	·1	5·8	5·0	5·1	4·6	0	0	0	0
kb	F21		—	—	0	—	+	6·1	·1	5·0	5·1	4·6	·1	0	0	0	0
kc	F22		—	—	0	—	+	5·9	·1	6·1	6·2	0	·2	·1	3·9	·1	0
kd	F23	C	—	—	0	—	+	6·1	·1	6·5	5·2	4·8	·1	0	0	0	0
ke	F23	E	—	—	0	—	+	5·9	·1	5·9	5·0	5·3	0	0	0	0	0
kf	F23	G	—	—	0	—	+	6·6	·2	0	5·0	4·4	6·3	0	·1	0	0
kg	F24	C	—	—	0	—	+	5·8	·1	5·9	5·2	4·3	·1	0	4·0	0	0
kh	F24	H	—	—	0	—	+	6·6	·1	6·0	5·3	4·7	0	·2	4·1	0	0
ki	F24	E	—	—	0	—	+	5·9	·1	5·5	5·9	5·2	4·3	·1	0	·2	0
kj	F25	C	—	—	0	—	+	4·9	0	5·2	4·3	4·8	·1	·1	4·1	2·3	·1
kk	F25		—	—	0	—	+	6·5	·2	5·4	4·9	4·8	6·2	0	·1	0	0
kl	F26		—	—	0	—	+	5·8	·2	5·9	5·4	5·1	0	0	0	·1	0
km	F26	E	—	—	0	—	+	6·1	·2	6·4	5·4	4·6	6·5	0	0	·1	0
kn	F26	D	—	—	0	—	+	5·8	·2	6·1	5·5	5·1	0	0	0	·1	·1
ko	F27	D	—	—	0	—	+	7·3	·2	5·9	5·7	·2	·2	0	2·2	·3	·1
kp	F28	D	—	—	0	—	+	7·5	·2	7·2	4·8	·5	·2	·3	1·7	·1	0
kq	F28	E	+	—	0	—	+	5·8	0	3·8	5·7	5·0	0	·2	0	·1	0
kr	F29		—	—	0	—	+	5·9	0	6·2	5·4	4·9	·1	·1	0	·1	0
ks	F30		—	—	0	—	+	5·2	·1	5·7	5·4	5·1	3·8	5·9	·1	·1	·1
kt	F31	E	—	—	0	—	+	5·5	·1	4·8	5·0	4·6	5·9	6·2	0	·1	0
ku	F31		—	—	0	—	+	5·9	0	5·1	5·0	5·7	5·6	0	·1	0	0
kv	F31		—	—	0	—	+	6·0	·1	5·4	4·7	0	0	0	0	0	0
kw	F31		—	—	0	—	+	5·4	0	5·5	5·3	0	0	0	0	·1	·2
kx	F32	C	—	—	0	—	+	5·1	0	4·3	5·0	5·2	6·3	5·8	0	0	0
ky	F33		—	—	0	—	+	4·9	·1	5·8	5·2	4·6	·2	0	0	·2	0
la	F34	G	+	36	0	—	+	5·1	·5	4·8	3·5	·5	·5	·7	4·6	2·3	·1
lb	F35	C	+	0	0	—	+	6·2	0	6·5	6·0	4·5	6·1	6·4	4·5	·1	0
lc	F35	C	+	0	0	—	+	6·6	0	6·4	1·7	4·7	·1	0	4·3	·1	0
ld	F36	E	—	—	0	—	+	5·2	0	5·2	5·0	4·6	6·1	6·1	0	·1	0
le	F36	H	+	—	0	—	+	6·3	0	4·9	4·7	4·6	·2	·1	·2	0	0
lf	F36	H	+	—	0	—	+	6·5	0	5·0	5·0	4·8	0	0	·1	0	0
lg	F37	D	—	—	0	—	+	5·7	0	5·0	4·6	4·4	6·0	6·4	·1	0	0
lh	F37		—	—	0	—	+	5·4	·1	5·2	5·6	4·3	6·0	·2	0	0	0
li	F37		—	—	0	—	+	6·1	0	4·9	5·1	4·9	5·9	6·3	·1	·1	0
lj	F38	E	+	0	0	—	+	6·3	0	5·2	4·9	4·8	6·4	·1	0	0	0
lk	F39	E	—	—	0	—	+	6·6	0	6·3	5·2	4·8	0	0	·2	0	0
ll	F39	E	+	—	0	—	+	6·8	0	6·2	5·1	5·0	0	0	3·9	0	0
lm	F39	E	—	—	0	—	+	6·5	0	6·4	4·7	4·4	0	·2	0	0	0
ln	F40	E	—	—	0	—	+	5·6	0	5·2	4·7	5·3	5·3	·1	·1	0	0
lo	F40	G	—	—	0	—	+	5·8	0	5·3	5·6	6·0	6·2	0	0	0	0
lp	F40		—	—	0	—	+	5·7	0	5·2	5·8	5·3	6·1	·2	3·6	0	0

TABLE II.—*Physiological characters of all cultures*—Continued.

Culture No.	Origin.	Morphology.	Chains.		Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid.									
			Milk.	Broth.				Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
kr	F41	E	...	...	99 mm.	...	...	6.2	0	5.5	5.2	5.2	6.1	0.1	0	0	0.1
ky	F41	E	...	...	0	...	...	6.8	.1	5.3	5.1	0	6.3	.1	0	.2	.1
kz	F41	E	...	...	0	...	...	6.5	.1	5.1	5.0	5.0	6.3	0	.1	.1	0
ma	F42	C	...	...	0	...	...	6.1	0	4.3	5.4	3.4	.2	3.9	4.4	.4	0
mc	F43	E	...	...	0	...	...	6.5	0	5.3	4.6	5.2	6.0	6.2	0	.1	0
md	F43	...	...	...	...	...	...	5.6	0	5.0	4.6	5.3	6.2	6.2	.1	.1	0
me	F44	C	...	...	0	...	...	6.6	0	5.2	4.8	5.1	7.0	0	0	0	0
mf	F45	C	...	...	0	...	...	6.6	.2	5.3	5.0	5.5	4.6	.1	0	0	0
mg	F46	E	...	...	0	...	...	5.4	0	4.8	4.6	4.6	6.5	.2	.1	0	0
mh	F46	E	...	...	0	...	...	5.9	0	5.4	4.6	4.3	0	0	.1	0	0
mi	F38	E	...	...	...	...	...	6.4	0	5.1	5.0	4.5	6.5	.1	.6	0	0
mj	F38	E	...	...	0	...	...	6.3	0	5.1	4.6	4.9	.1	0	0	.2	0
mk	F47	D	...	...	0	...	...	6.4	0	4.8	5.1	5.0	6.2	.2	.2	0	0
ml	F47	...	...	...	...	...	...	6.0	0	5.0	4.9	4.9	6.1	5.6	0	0	0
mm	F47	C	...	...	0	...	...	6.4	0	4.7	5.3	5.2	6.3	5.8	0	.2	0
mn	F48	E	...	...	...	...	...	5.6	0	5.9	5.5	.1	.1	.3	1.5	0	.1
mo	F48	...	...	...	0	...	...	5.7	0	5.9	5.0	0	.1	.2	3.0	.4	0
mq	F49	E	...	...	0	...	...	6.2	0	5.3	5.3	5.5	6.2	6.5	0	.2	0
mr	F49	E	...	...	0	...	...	6.6	0	5.1	4.8	5.1	6.2	5.7	.1	0	.1
ms	F50	E	...	...	0	...	...	6.4	0	5.1	4.9	0	6.1	0	0	0	0
mt	F50	A	...	+	0	...	...	6.1	0	5.2	5.0	5.0	6.4	6.2	0	0	0
mu	F51	A	...	+	0	...	...	6.0	0	5.1	5.0	5.0	6.4	0	0	0	0
mv	F51	H	...	...	0	...	...	5.9	.2	5.1	4.7	5.2	6.2	6.0	0	0	.1
mw	F52	E	...	...	0	...	...	5.7	0	5.0	4.8	5.4	6.2	5.5	.7	0	0
mx	F52	...	...	...	0	...	...	5.9	.1	3.8	5.0	0	.1	0	0	.1	0
my	F53	...	...	...	0	...	...	5.5	.1	5.2	4.9	4.9	6.1	5.9	.1	0	.4
mz	F53	E	...	...	0	...	...	5.8	.2	3.9	4.8	0	.2	.1	.2	.1	0
na	F53	E	...	...	0	...	...	6.5	0	5.0	5.2	5.4	5.3	0	0	0	0
nb	F54	...	...	...	0	...	...	5.0	0	5.1	4.7	0	0	0	.1	0	0
nc	F54	...	...	...	...	...	...	4.4	.2	5.1	4.9	0	0	0	0	.1	.1
nd	F55	...	...	...	...	...	...	5.6	0	5.9	5.4	5.3	4.5	0	0	0	.1
ne	F55	...	...	...	0	...	...	5.9	.2	6.0	5.4	5.5	.2	.2	0	.2	0
nf	F56	...	...	...	0	...	...	5.8	.1	5.1	5.1	0	.2	0	0	0	.1
ng	F56	...	...	...	0	...	...	4.7	.1	5.2	5.5	0	0	0	0	.2	.1
nh	U9	H	...	...	0	...	...	4.6	0	4.6	3.1	.1	.2	0	.1	.3	0
ni	U9	...	...	...	0	...	...	4.9	0	5.2	5.0	.2	0	.1	0	.3	.1
nk	U9	B	...	...	0	...	...	5.1	.1	4.8	5.1	.2	.2	0	.1	.1	.1
nl	U10	G	...	...	35	...	...	7.2	.1	4.7	5.1	.2	.4	.3	4.9	1.8	.1
nm	U10	G	...	...	24	...	...	7.4	0	5.1	4.3	.2	.5	.1	5.1	1.8	.3
nn	U10	H	...	+	32	...	...	7.4	.2	4.4	5.1	.2	3.0	.2	5.2	1.7	.2
no	U11	G	...	+	0	...	...	4.4	0	4.7	3.8	0	.5	0	.2	.3	.1
np	U12	H	...	+	0	...	...	5.8	0	0	.4	0	...	0	4.5	.4	0
nq	U13	H	...	+	0	...	...	5.3	0	4.1	4.7	.1	0	.1	0	2.0	0
nr	U14	B	...	+	0	...	...	6.4	0	5.6	1.5	0	0	0	5.5	.5	.1
ns	U14	E	...	+	0	...	...	6.4	0	5.5	5.1	0	0	0	4.8	.3	.1
nt	U15	H	...	+	0	...	...	5.8	0	5.1	4.8	.2	0	0	.1	1.7	0
nu	U16	H	...	+	0	...	...	3.8	0	4.4	4.7	.2	0	.1	.3	0	.1
nv	U16	H	...	+	0	...	...	5.9	0	4.7	4.8	.2	.1	0	.2	.1	0
nw	U16	H	...	+	0	...	...	5.4	0	4.4	4.7	.3	1.1	0	.1	.9	0
nx	U17	H	...	+	0	...	...	5.9	.2	4.0	4.5	.2	0	.1	.4	.4	.2
ny	U17	H	...	+	0	...	...	5.2	.1	.1	4.5	.3	.2	0	.2	.5	.1
nz	U17	H	...	+	0	...	...	6.0	0	4.5	4.6	.3	0	.1	.1	0	.1
oa	B1	C	...	...	0	...	...	5.7	.4	4.4	5.2	5.6	4.8	5.5	.1	0	.2
ob	B1	...	...	...	0	...	...	4.4	0	5.3	4.9	4.4	4.1	0	0	0	.2
oc	B2	...	...	...	0	...	...	4.3	0	5.4	4.7	4.5	.1	0	0	0	.1
od	B2	F	...	...	0	...	...	5.7	0	4.6	4.5	7.1	0	3.0	4.0	.2	0
oe	B3	F	...	...	0	...	...	5.4	0	4.9	5.0	4.1	0	3.3	3.8	.1	0
of	B3	F	...	...	0	...	...	5.8	0	4.5	4.6	4.0	0	3.3	3.9	.1	0
og	B4	F	...	...	0	...	...	5.4	0	4.0	5.1	3.9	0	3.0	3.8	.5	0
oh	B5	E	...	+	0	...	...	6.1	0	6.2	5.5	.1	.1	0	4.6	.3	0
oi	B6	E	...	...	0	...	...	5.9	0	5.8	5.0	1.7	0	3.7	3.9	.2	0
oj	B7	E	...	...	0	...	...	5.7	.2	4.4	5.4	3.8	0	0	3.8	.3	0
ok	B7	E	...	...	0	...	...	5.4	0	1.4	4.8	4.3	.3	0	3.9	.4	.1
ol	B8	G	...	...	0	...	...	6.1	.1	.2	5.4	0	0	0	4.3	.4	.1
om	B8	E	...	...	0	...	...	6.4	.1	.2	5.5	0	.1	0	4.2	.2	.1
on	B9	E	...	...	0	...	...	6.0	0	.2	5.3	0	.1	0	4.5	0	0
oo	B9	E	...	...	0	...	...	6.3	.1	.2	5.5	.2	0	0	4.4	.1	.1
op	B10	E	...	...	0	...	...	6.1	0	6.1	5.2	.1	.2	0	4.6	.2	.2
oq	B10	E	...	...	0	...	...	6.6	0	7.1	5.5	6.2	5.0	0	.1	.1	.2
or	B11	E	...	...	0	...	...	6.0	0	6.0	5.4	0	.1	0	4.4	.1	.2
os	B11	E	...	...	0	...	...	6.1	0	5.9	2.0	.1	0	0	4.6	.3	.2
ot	B12	G	...	...	0	...	...	6.9	.1	5.9	5.1	.1	0	0	0	.3	.2
ou	B12	G	...	...	0	...	...	6.8	.1	5.9	5.1	.1	.1	0	3.8	.2	.2
ov	B13	G	...	...	0	...	...	6.3	.1	6.1	5.3	0	0	.1	4.0	.3	.2

TABLE II.—*Physiological characters of all cultures*—Continued.

Culture No.	Origin.	Morphology.	Chains. Milk.	Chains. Broth.	Liquefied gelatin.	Neutral red reduction.	Milk curd.	Percentage of normal acid. Dextrose.	Adonite.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Dulcite.
					mm.												
oy	Bt3	C	....	—	0	—	—	6.6	0.1	5.8	5.6	0.1	0	0	3.7	0.2	0.1
oz	Ut8	B	....	+	0	—	+	4.9	.1	.4	4.5	.1	.1	.1	0	0.	.2
pa	Ut8	....	....	....	0	—	+	5.2	0	.1	4.3	0	0	0	0	.1	.1
pb	Ut9	....	....	....	0	—	+	4.9	0	4.6	5.3	.2	0	0	0	.2	.2
pc	Ut9	....	....	....	0	—	+	5.0	0	5.2	5.3	.1	0	.2	.1	.1	.1
pd	Bt4	E	....	—	0	—	+	4.8	.2	6.2	4.5	.1	0	0	3.9	.2	0
pe	Bt4	E	....	—	0	—	+	4.3	0	4.1	4.6	0	0	0	3.5	.1	0
pf	Bt5	E	....	—	0	—	+	5.3	0	4.4	5.0	3.8	0	2.8	3.7	.2	0
pg	Bt5	E	....	—	0	—	+	5.4	0	4.5	5.0	3.9	0	2.9	3.6	.3	0
ph	Bt6	G	....	—	0	—	+	6.0	.2	1.8	4.5	.1	0	0	4.3	.1	.2
pi	Bt6	G	....	—	0	—	+	5.9	.3	1.8	4.7	.1	0	0	4.7	.2	.1
pj	Bt7	E	....	—	0	—	+	4.7	0	4.1	4.7	0	.1	0	3.5	.1	0
pk	Bt7	E	....	....	....	—	+	4.6	0	4.2	4.8	0	0	0	3.9	.2	.3
pl	Bt8	E	....	—	0	—	+	5.5	0	4.2	5.0	3.9	0	2.8	3.7	.5	.1
pm	Bt8	E	....	—	0	—	+	5.6	0	4.2	4.8	4.0	0	2.8	3.8	.4	0
pn	Bt9	C	....	—	0	—	+	4.8	0	4.1	4.6	3.3	0	0	3.6	.2	0
po	Bt9	E	....	—	0	—	+	4.8	0	4.3	4.8	3.2	0	0	3.8	.1	.1
pq	Bt10	E	....	—	0	—	+	5.2	0	4.2	4.8	0	.2	.2	3.6	1.6	.2
pr	Bt10	E	....	—	0	—	+	5.4	.1	4.3	4.8	0	0	.3	3.5	.1	.2
ps	Bt11	E	....	—	0	—	+	6.2	.1	4.2	5.1	0	0	.1	2.9	0	0
pt	Bt11	E	....	—	0	—	+	6.2	0	5.3	4.9	0	0	.2	2.9	0	0

In one particular our results do not agree with the conclusions reached by Stowell, Hilliard, and Schlesinger[1] and by Howe and others in that the "metabolic gradient" which they establish, in our opinion, can be correct only for the particular group under consideration, since the number of cultures utilizing any particular carbohydrates or similar compound is dependent on the peculiarities of the cultures as well as on the composition or the configuration of the test substance. While in a general way our cultures follow the scheme outlined by Stowell, Hilliard, and Schlesinger, this arrangement may be varied, as will be pointed out later, by varying the source from which the cultures are obtained. In one group of our collection a much larger percentage of cultures give a fermentation with mannite than with raffinose; in others the conditions are reversed. In no case did we obtain a higher percentage of positive results with mannite than with inulin, although both Winslow and Stowell, Hilliard, and Schlesinger put inulin above mannite. Dulcite may be considered as one of the more difficultly fermented alcohols, and yet in our work on the colon group we found that dulcite was fermented most frequently, not by the more active group but by the one which otherwise showed weak fermentative ability. With adonite the conditions were reversed.

There is among all acid-forming bacteria and especially among the streptococci considerable variation in the maximum amount of acid produced. Winslow has shown that this may be a valuable aid in dis-

[1] Stowell, E. C., Hilliard, C. M., and Schlesinger, M. J. A statistical study of the streptococci from milk and from the throat. Jour. Infect. Diseases, v. 12, no. 2, p. 144–164. 1913.

tinguishing cocci of different species.[1] Stowell, Hilliard, and Schlesinger, in the paper already quoted,[2] have pointed out the marked difference in this regard between streptococci from milk and those from the human throat. In Table III is shown the distribution of cultures according to their source and the quantity of acid formed in dextrose broth. This is also shown graphically in figure 4. The mode for the culture from the mouth falls over 6.5 per cent, while that for the udder organisms is over 5.0 per cent, and that for those from feces is 5.5 per cent. The mode for each group is sharply defined, especially those for the udder and feces groups. On the assumption that the cultures obtained from milk may have come originally from any of the other sources, we would expect the curve representing the milk cultures to spread over the space occupied

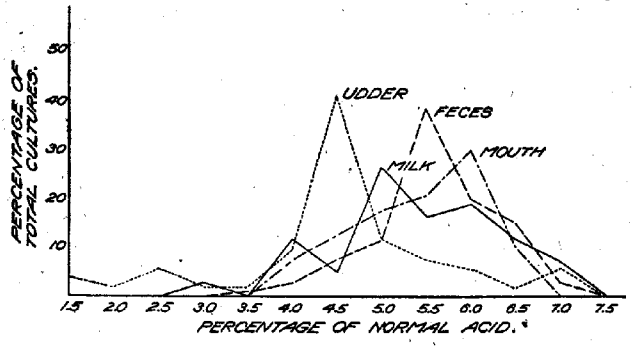


FIG. 4.—Frequency curves showing acid formation in dextrose broth.

by the other curves. This is true in a general way, but the curve for the milk cultures has a mode falling between that for the udder and the feces cultures. It should be remembered that the milk cultures were not selected promiscuously but from bile tubes incubated at 37° C.

TABLE III.—*Distribution of cultures according to the percentage of normal acid produced in dextrose broth.*

Source.	Total number of cultures.	Below 1.0.	1 to 1.5.	1.5 to 2.0.	2.0 to 2.5.	2.5 to 3.0.	3.0 to 3.5.	3.5 to 4.0.	4.0 to 4.5.	4.5 to 5.0.	5.0 to 5.5.	5.5 to 6.0.	6.0 to 6.5.	6.5 to 7.0.	7.0 to 7.5.	Above 7.5.
Milk:																
Number	42	0	0	0	0	0	1	0	5	2	11	7	8	5	3	0
Per cent		0	0	0	0	0	2.38	0	11.90	4.76	26.19	16.67	19.05	11.90	7.14	0
Udders:																
Number	51	0	0	2	1	3	1	1	5	21	6	4	3	1	3	0
Per cent		0	0	3.92	1.96	5.88	1.96	1.96	9.80	41.18	11.76	7.84	5.88	1.96	5.88	0
Feces:																
Number	114	0	0	0	0	0	0	1	3	9	13	44	23	18	3	0
Per cent		0	0	0	0	0	0	0.88	2.63	7.89	11.40	38.59	20.17	15.79	2.63	0
Mouth:																
Number	39	0	0	0	0	0	0	0	3	5	7	8	12	4	0	0
Per cent		0	0	0	0	0	0	0	7.69	12.82	17.95	20.51	30.77	10.26	0	0

[1] Winslow, C. E. A., and Winslow, Anne R. Systematic relationships of the Coccaceae. ed. 1, 300 p., illus. New York, 1908.

[2] Stowell, Hilliard, and Schlesinger. Op. cit.

ACTION ON LITMUS MILK

Late in the course of the investigation it was noticed that there were distinct differences in the action of different cultures on the litmus in milk and that this difference was in some relation to the source of the cultures. Some cultures decolorized the litmus promptly, leaving a white curd, with the exception of a pink ring at the top, which slowly extended downward. Other cultures produced a curd which remained pink throughout for an indefinite period. This action was recorded for the cultures then available, and the results are given in Table IV. It will be noticed that while the ability to reduce litmus is characteristic of the mouth cultures it is almost entirely lacking in the cultures from

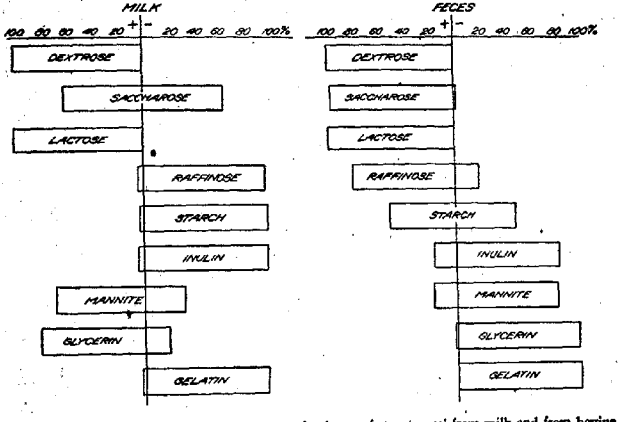


FIG. 5.—Graphic representation of the characters of cultures of streptococci from milk and from bovine feces.

the udder. The number of cultures in the two other groups in which this character was recorded is too small to permit conclusions, but there may be observed a tendency in the milk cultures to agree with those from the udder.

TABLE IV.—*Distribution of cultures according to action on litmus in milk.*

Cultures recorded from—	Number of cultures.	Cultures reducing litmus.		Cultures failing to reduce litmus.
		Number.	Percent.	
				Per cent.
Milk	17	4	23.53	76.57
Feces	16	6	37.50	62.50
Udder	29	2	6.89	93.10
Mouth	35	29	82.86	17.14

THE FERMENTATION OF TEST SUBSTANCES

In Table V the cultures are arranged on the basis of fermentation or nonfermentation of eight test substances. In this table all reactions of 1 per cent or over are counted as positive and those falling below as negative. The results given in this table are arranged in a form more easily studied in figures 5 and 6. In these diagrams all positive results are plotted to the left of a vertical line and the negative results to the right. The udder organisms are characterized by the general lack of ability to ferment the test substances. They fail almost without exception to ferment raffinose and the polysaccharids, but show some tendency to attack the two alcohols. On the other hand, the 114 cultures

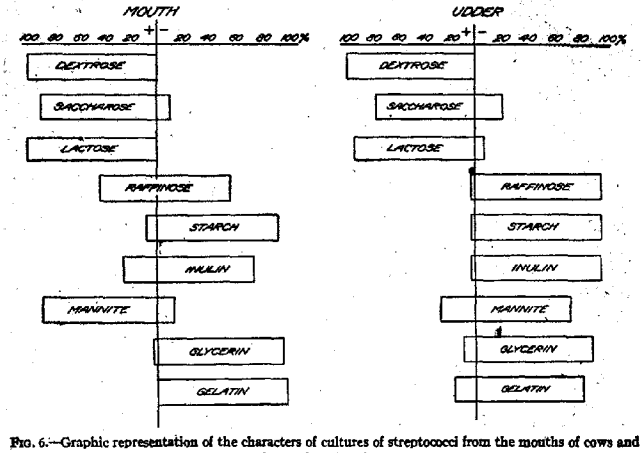


FIG. 6.—Graphic representation of the characters of cultures of streptococci from the mouths of cows and from infected udders.

from bovine feces fail almost entirely to utilize the alcohols, but show exceptional activity in fermenting the more complex sugars and the polysaccharids.

TABLE V.—*Fermentation of test substances.*

Origin of culture.	Dextrose.		Saccharose.		Lactose.		Raffinose.		Starch.		Inulin.		Mannite.		Glycerin.	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Milk:																
Total.....	42	0	21	21	42	0	2	40	1	41	2	40	29	13	34	8
Percentage of total....	100	0	50	50	100	0	4.8	95.2	2.3	97.7	4.8	95.2	69.0	31.0	80.9	19.1
Udder:																
Total.....	51	0	40	11	48	3	0	51	2	49	2	49	14	37	6	45
Percentage of total....	100	0	78.4	21.6	94.3	5.7	0	100	4	96	4	96	27.4	72.6	11.6	88.4
Feces:																
Total.....	114	0	112	2	114	0	93	21	60	54	20	94	21	93	2	112
Percentage of total....	100	0	98.2	1.8	100	0	81.5	18.5	52.5	47.5	17.6	82.4	18.5	81.5	1.8	98.2
Mouth:																
Total.....	40	0	35	4	39	0	17	22	3	36	10	29	34	5	1	38
Percentage of total....	100	0	89.7	11.3	100	0	43.6	56.4	7.7	92.3	25.6	74.4	87.2	12.8	2.6	97.4

The cultures from the mouth differ from those from the udder in the higher percentages of raffinose, inulin, and mannite fermenters and in less action on glycerin and gelatin. They are sharply differentiated from the feces organisms in their general failure to ferment starch and the much higher percentage of mannite fermenters.[1]

The milk cultures are distinguished by the comparatively small number of saccharose fermenters, the failure to ferment raffinose, starch, and inulin, and the active fermentation of both mannite and glycerin.

THE LIQUEFYING CULTURES

It will be noted that with the exception of a few obtained from milk, all of the liquefying cultures came from the udder. If we consider the 11 gelatin-liquefying cultures as a group we obtain the data given in Table VI, which shows that the liquefaction of gelatin is not an isolated variation from the type but is correlated with an ability to utilize the alcohols, mannite, and glycerin. This peculiar correlation between gelatin liquefaction and glycerin fermentation was also noticed in the colon group.

TABLE VI.—*Comparison of liquefying and nonliquefying cultures of streptococci from the udder.*

Item.	Gelatin.	Dextrose.		Saccharose.		Lactose.		Raffinose.		Starch.		Inulin.		Mannite.		Glycerin.	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Number of cultures	+	11	0	10	1	11	0	0	11	1	10	0	11	9	2	6	5
Per cent		100.0	0	90.90	9.09	100.0	0	0	100.0	9.09	90.90	0	100.0	81.81	18.19	54.54	45.46
Number of cultures	−	43	0	33	10	40	3	0	43	2	41	2	41	7	36	0	38
Per cent		100.0	0	76.74	23.26	93.02	6.98	0	100.0	4.65	95.35	4.65	95.35	16.28	83.72	0	100.0

The characters of the 11 cultures included in Table VI agree very closely with the ' Group C" of the article by the writers on the lactic-acid bacteria.[2] If we divide the udder cultures into gelatin-liquefying and nonliquefying groups, we obtain figure 7, in which the cultures are arranged as in figures 5 and 6. This gives two groups in each of which the cultures show distinctive characters and remarkable uniformity.

We have, then, at least three sharply defined varieties: Two from the udder, of which one has weak fermentative ability, attacking only dextrose, saccharose, and lactose, with an occasional culture-producing acid from mannite, inulin, or starch, and a second less numerous type, which liquefies gelatin and ferments dextrose, saccharose, lactose, mannite, and usually glycerin; and one from bovine feces, character-

[1] Since this paper was written, a communication by C. A. Fuller and V. A. Armstrong entitled "The differentiation of fecal streptococci by their fermentative reactions in carbohydrate media" has appeared in the Jour. of Infect. Diseases, v. 13, no. 3, p. 442-462, Nov., 1913. The characteristics of their cultures from bovine feces agree in all essential particulars with those found by the writers.

[2] Rogers, L. A., and Davis, B. J. Methods of classifying the lactic-acid bacteria. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 154, 30 p., 6 fig. 1912.

ized by its active fermentation of sugars and polysaccharides and general failure to ferment the alcohols, mannite and glycerin. The group from the mouth has certain distinctive characters, but is not as clearly defined as the other three groups. It will need additional study before it can be described as a distinct variety.

If we consider the milk cultures individually, we find that two of them, *ik* and *il*, clearly belong with the feces group. The one which liquefies gelatin has the characters of the typical liquefying udder culture. The remaining 39 cultures may be placed with the nonliquefying udder organisms. If, however, we assume that the fermentation of mannite and glycerin places the nonliquefiers with the liquefiers, an assumption

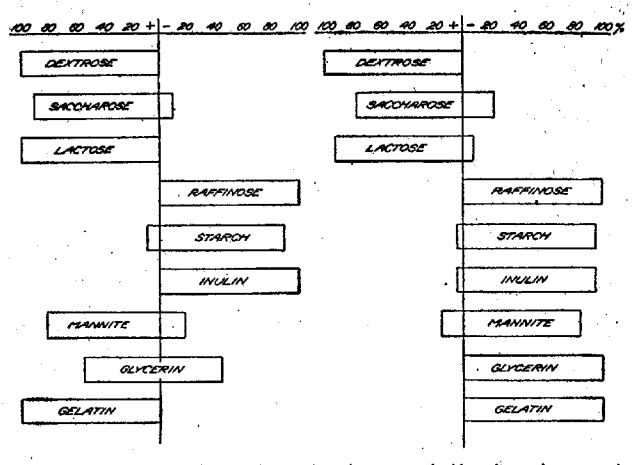


FIG. 7.—Diagram showing the fermentation reactions of two types of udder cultures of streptococci.

based on the possible variation of the liquefying power, we obtain a division of the milk cultures as shown in Table VII and figure 8. Thus, we obtain two groups agreeing very closely with those into which we were able to separate the udder cultures. This points very strongly to the infected udders rather than to the feces as the source of chain-forming cocci growing in lactose broth at 37° C.

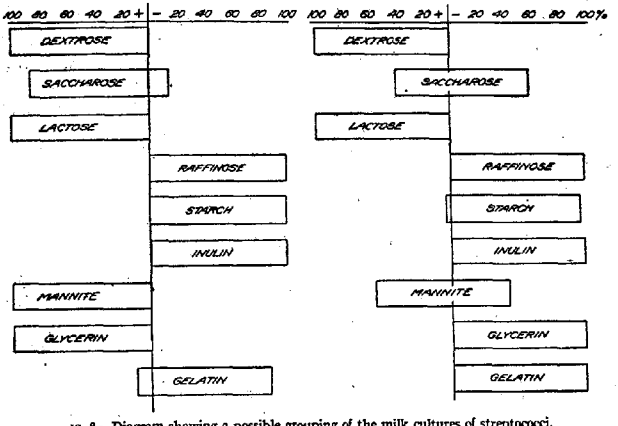
TABLE VII.—*Two possible groups of the milk cultures.*

Significant characters.	Total number of cultures.	Gelatin. +	Dextrose. +	Saccharose. +	Lactose. +	Raffinose. +	Starch. +	Inulin. +	Mannite. +	Glycerin. +
Gelatin +.....	.....	1	8	7	8	0	0	0	8	8
Mannite +.....	8	.....	.....	.....	.....	.....	.....	.....	.....	.....
Glycerin +..per ct..	.....	12.5	100	87.5	100	.....	.....	.....	100	100
Gelatin −.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mannite +.....	32	0	32	13	32	0	1	0	18	0
Glycerin −..per ct..	.....	.....	100	40.6	100	.....	3.1	.....	56.3	.....

The same test applied to the mouth cultures would show that almost any individual culture could be included in the feces group. However, almost any mouth culture would be an exceptional, not a typical, feces culture. A culture fermenting saccharose, lactose, raffinose, and mannite could be either from the mouth or from feces, but there is a high probability that it would be of buccal origin. On the other hand, a culture fermenting saccharose, lactose, raffinose, and starch, but failing to ferment mannite or glycerin, would almost certainly be of fecal origin.

RELATION OF THESE GROUPS TO NAMED VARIETIES

It would be difficult to identify all of these groups with previously described species. Until the work of Gordon, few cultures were described



IG. 8.—Diagram showing a possible grouping of the milk cultures of streptococci.

on the basis of the fermentation of a large number of test substances, and in only a very few cases have the cultures been obtained from a definite source. An exception may be made of the pathogenic bacteria in which the cultures described have been selected from definite and very similar sources. Among the streptococci we have an example in the pus-forming organism generally described as *Streptococcus pyogenes*. In Table VIII are compiled the typical reactions given for *Streptococcus pyogenes* by three investigations. The reactions given by Andrewes and Horder are compiled from a large number of cultures, and those given by Gordon are from a number of his own cultures.[1] Those given by Bergey are the reactions of a comparatively few typical cultures.[2] So

[1] Andrewes, F. W., and Horder, T. J., A study of the streptococci pathogenic for man. Lancet, v. 2, no. 11, p. 708–713; no. 12, p. 775–782; no. 13, p. 852–855. 1906.

Gordon, M. H. Report on an investigation of the fermentative characters of streptococci present on fauces during scarlet fever. 40th Ann. Rpt. Local Govt. Bd. [Gt. Brit.], 1910–11, Suppl. Rpt. Med. Off., p. 302–31, 1911.

[2] Bergey, D. H. Differentiation of cultures of streptococcus. Jour. Med. Research, v. 27 (n. s., v. 12), no. 1, p. 67–77. 1912.

far as it is possible to make comparisons, the reactions given agree very closely with our nonliquefying udder cultures.

TABLE VIII.—*Results of fermentation tests of Streptococcus pyogenes described in the literature.*

Authority.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Salicin.	Conferin.
Our nonliquefying udder cultures...per cent..	76	93	0	4	4	16	16	.....	.....
Andrewes and Horder.....	+	+	—	.....	—	±	.....	—	—
Gordon.....	+	+	—	.....	—	.....	.....	—	.....
Bergey.....	+	+	—	.....	—	.....	.....	—	.....

A still further comparison is possible by the tabulation of the fermentation reactions of five typical cultures of *Streptococcus pyogenes* obtained through the courtesy of Prof. C. E. A. Winslow, of the American Museum of Natural History. These results are given in Table IX. Although some of these cultures have been grown on artificial media for many years, they still exhibit the same general characters as our freshly isolated udder cultures—namely, an ability to ferment dextrose, saccharose, and lactose, general failure to ferment raffinose and the polysaccharids, and an erratic tendency to ferment the alcohols. Unfortunately the gelatin test was not made on these five cultures. The fermentation of glycerin by three of the five indicates that they may have been of the liquefying type. Savage in 176 cultures of streptococci isolated from cases of mastitis found that 95 per cent liquefied gelatin.[1] His cultures differed from both the typical *S. pyogenes* and our liquefying cultures in that 49 per cent fermented raffinose.

TABLE IX.—*Results of fermentation tests of five cultures of Streptococcus pyogenes from American Museum of Natural History (New York) collection.*

Source.	Dextrose.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.
New York Post Graduate Medical College (fatal septicemia).....	3.90	3.85	3.60	0.20	0.18	0.23	6.35	0.59
Dr. Bien, Chicago, Ill. (abscess in erysipelas).....	5.45	4.75	3.95	0	.13	.18	2.55	2.34
Boston Board of Health (urine).....	3.85	4.05	.45	0	3.98	0	0	1.74
Johns Hopkins University.....	6.45	4.95	4.70	.20	.45	.08	4.91	1.67
Michigan Agricultural College.....	2.50	0	1.15	.05	0	.09	.23	.19

VARIATION FROM TYPE IN THE UDDER ORGANISMS

The trouble from infected udders at both the Beltsville and Annapolis farms was in the nature of an epidemic. The infection apparently spread from cow to cow and became so severe that at Annapolis one or

[1] Savage, W. G., Report upon the bacteriology and pathology of "Garget" (or mastitis) in cows. 37th Ann. Rept. Local Govt. Bd. [Gt. Brit.], 1907-8. Suppl. Rept. Med. Off., pp. 359-424. 1909.

more cows were rendered useless. There was no apparent connection between the two epidemics except that they occurred at about the same time. We may assume that these epidemics originated in one of two ways, either of which must admit more or less variation in physiological reactions from the original type. It may be possible that the udders of one or more cows may have become infected by some of the streptococci coming originally from the mouth, intestines, or other sources. Under the influence of its new environment this organism may have acquired pathogenic properties sufficient to produce the symptoms observed in mammitis. Heinemann has shown that pathogenicity is a property readily acquired when ordinary streptococci are grown in animals.[1] If these infecting organisms came from the mouth, the intestines, or the milk they must have acquired in a comparatively short time an entirely new set of biochemical reactions in addition to a variation in pathogenicity. On the other hand, we may assume with much more appearance of reasonableness that the infection spread from a single infecting cell or aggregate of similar cells which already possessed pathogenic powers and general characters identical with those we have found to be characteristic of the udder organisms. This assumption is in accord with the established fact that streptococci from pathological lesions in general have similar biochemical reactions. If the infection in these two cases came from various sources, it must follow that growth under similar conditions would produce uniform fermentation reactions in a short time, a view held by Walker, who maintains that these reactions may be varied almost at will and can only indicate the latest habitat of the culture.[2] If the infection came from a single cell, there must have been some variation, since the fermentation reactions were not identical at the time of this isolation.

In Table X are shown the varieties of nonliquefying udder cultures and the number occurring in each of the two herds. There were seven varieties in all. The most numerous one ferments dextrose, saccharose, and lactose only and occurred 24 times, equally divided between the two herds. The next most numerous variation differed from the first in failing to ferment saccharose and occurred 8 times. A third variation fermented mannite in addition to dextrose, saccharose, and lactose and occurred 4 times. The remaining four varieties evidently occur only once or twice in every 40 cultures. Viewed from any standpoint it is evident that these organisms are subject to variation from the type, but these variations are not of sufficient magnitude or frequency to detract from the value of the physiological reactions as a means of establishing true species.

[1] Heinemann, P. G., The pathogenicity of *Streptococcus lacticus*. Jour. Infect. Diseases, v. 4, no. 1, p. 87–92. 1907.

[2] Walker, E. W. A., On variation and adaptation in bacteria, illustrated by observations upon streptococci, with special reference to the value of fermentation tests as applied to these organisms. Proc. Roy. Soc. [London], s. B, v. 83, no. 567, p. 541–558. 1911.

TABLE X.—*Variation from type in nonliquefying udder cultures.*

Significant characters.								Number of cultures from herd at—		Total number of cultures.
Dextrose.	Saccharose.	Lactose.	Raffinose.	Starch.	Inulin.	Mannite.	Glycerin.	Beltsville.	Annapolis.	
+	+	+	−	−	−	−	−	12	12	24
+	−	+	−	−	−	−	−	6	2	8
+	+	+	−	−	−	+	−	2	2	4
+	+	+	−	−	+	−	−	1	1	2
+	+	+	−	−	−	−	+	0	1	1
+	+	+	−	−	−	+	−	1	0	1
+	+	+	−	−	+	+	−	1	0	1

SUMMARY

A collection of cultures of streptococci was made consisting of 42 cultures from milk which formed chains in lactose bile at 37° C., 51 cultures from infected udders, 114 cultures from bovine feces, and 39 cultures from the mouths of animals.

The morphology varied under different conditions and could not be correlated with the source of the culture, except that the udder cultures had a more marked tendency to chain formation than those from other sources.

The ability of these cultures to liquefy gelatin and to form acid from dextrose, lactose, saccharose, raffinose, starch, inulin, mannite, glycerin, dulcite, and adonite was determined. Only one or two cultures utilized adonite or dulcite.

When glycerin was attacked, the fermentation proceeded slowly, failing to reach its maximum in 14 days, in contrast to the fermentation of the sugars, in which the maximum was reached in two or three days.

A high percentage of the udder cultures failed to give the characteristic reduction in litmus milk.

Twelve cultures liquefied gelatin; one of these came from milk and 11 from infected udders.

The cultures from feces were characterized by their activity in fermenting the sugars, including raffinose, and their inability to utilize the alcohols.

The mouth cultures fermented dextrose, saccharose, lactose, mannite, and freqently raffinose, but were almost without effect on starch and glycerin.

The udder cultures were characterized by the general lack of fermentative ability, which was limited almost entirely to dextrose, saccharose, and lactose, with a comparatively small number utilizing mannite, glycerin, and gelatin.

When the udder cultures were divided on the basis of gelatin liquefaction, two groups were obtained. The fermentative activities of one

of these, which are similar to those of *Streptococcus pyogenes*, were limited to dextrose, saccharose, and lactose, with an occasional culture fermenting mannite, starch, or inulin. The second group fermented the three simple sugars, mannite, and usually glycerin and liquefied gelatin.

When the milk cultures were considered individually, it was found that with the exception of two which clearly came from feces they could be included in one or the other of the two groups into which the udder cultures were divided.

Of the 41 nonliquefying udder cultures 24 gave identical reactions. The remaining cultures differed from the type in one or two characters only.

PRELIMINARY AND MINOR PAPERS

CRYSTALLIZATION OF CREAM OF TARTAR IN THE FRUIT OF GRAPES

By WILLIAM B. ALWOOD,

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During the chemical examinations made of the ripening fruit of grapes in the Enological Laboratory, Charlottesville, Va., the writer was led to conclude that the acid salt bitartrate of potassium was deposited from the juice in quantity sufficient to sensibly affect the analytical results. This led to the preparation of samples by the complete exhaustion of the soluble constituents of the berries, with results which supported the above conclusion.

The question of the character and location of the crystals of cream of tartar in the berry presented itself as a matter of interest and possibly of practical importance. The literature available did not furnish specific information on this point. Babo and Mach, in their exhaustive treatise, give but one brief reference to the occurrence of this salt in crystals in the fruit.[1]

As soon as the fruit was well colored at Charlottesville in 1912 a series of microscopic examinations was undertaken to determine whether crystals of bitartrate of potassium occurred in the fruit. Portions of Concord grapes were prepared and examined daily until the fruit was ripe. Minute crystals varying much in shape and size were found in great abundance in the soft cells lying just beneath the skin of the fruit. Crystals were not present at any time in the pulp or compact portion of the flesh in which the seeds are contained. Like examinations of Concord and Catawba were carried on at Sandusky, Ohio, in September and October, 1912, and crystals of the same general type were found.

The fact that many of the crystals found in the berries did not conform in type to crystals of the bitartrate prepared from pure cream of tartar made it doubtful as to whether potassium bitartrate was deposited or not. Therefore, the fruit was separated into portions for the purpose of a chemical examination covering this point. The tough pulp containing the seeds of 1,500 grams of ripe berries was separated from the hulls and soft peripheral layer of cells which adhere to the hulls. This layer contains the coloring matter. The hulls and pulp were then carefully pressed by hand and the juice of each recovered and held separately. This gave three portions: (1) The pressed hulls, (2) the juice recovered from the hulls, and (3) the juice recovered from the pulp.

In preparing the sample all the juice possible was recovered from the sample of hulls and pulp; that is, they were entirely exhausted so far as crushing and pressing could accomplish this result. The pressed hulls were then carefully macerated in distilled water until the soluble organic matter was exhausted. These portions showed on analysis the results given in Table I.

[1] Babo, A. F., and Mach, E. Handbuch des Weinbaues und der Kellerwirtschaft. Aufl. 4, Bd. 2, p. 16. Berlin, 1910.

TABLE I.—*Analyses of Concord grapes in 1912, giving the percentage by weight of acids and acid salts.*

Portion analyzed.	Total acid.	Total tartaric acid.	Free tartaric acid.	Cream of tartar.
Hulls exhausted with water	0.429	[a]0.589	0.08	0.56
Juice pressed from hulls	.141	.054	.00	.07
Juice pressed from pulp	1.065	.724	.20	.59

[a] The results show for the samples of "hulls" a greater content of tartaric acid than the total titratable acid of the samples. This is always the case in grape samples where the "acids other than tartaric" fall below a certain proportion.

The results show that the juice pressed from the hulls is very low in acid and acid salts, and that, while the organic matter remaining in the hulls after pressure is less than half as acid as the pulp, it is rich in tartaric acid and cream of tartar, in these regards nearly equaling the percentage found in the juicy pulp. The actual weight of the pressed hulls was 304 grams, or one-fifth of the original sample of fruit. From the results given, it would appear that the hulls when pressed dry still retained the crystals observed with the microscope, and actual observation has demonstrated this fact. The results for tartaric acid and cream of tartar settle the point as to the composition of these crystals.

Analyses of like import were made at Sandusky, Ohio, of samples of Catawba and Concord grapes. The results show that the acid content of the soft layer of cells attached to the hulls is proportionally richer in tartaric acid and cream of tartar than the pulp.

In 1913 the microscopic examinations were begun much earlier, and four varieties of grapes were included—Delaware, Concord, Niagara, and Norton. The presence of crystals of bitartrate of potassium could be observed before the berries were all colored, and the analyses of partly ripe fruit confirm the results of 1912. These samples were separated into two portions only, the hulls and the pulp, as noted above; then each sample was completely exhausted of soluble organic matter by repeated macerations and heating in distilled water. Table II gives the results for one set of samples from each of two varieties.

TABLE II.—*Analyses of grapes in 1913, giving percentage by weight of acids and acid salts*

Portion analyzed.	Total acid.	Total tartaric acid.	Free tartaric acid.	Cream of tartar.
CONCORD.				
Hulls	0.95	[a]1.11	0	1.33
Pulp	1.43	.79	.04	.82
NIAGARA.				
Hulls	0.67	[a]0.93	0	1.18
Pulp	.96	.83	.18	.57

[a] The results show for the samples of "hulls" a greater content of tartaric acid than the total titratable acid of the samples. This is always the case in grape samples where the "acids other than tartaric" fall below a certain proportion.

There are crystals other than bitartrate present in the fruit, but this paper is intended only to record an observation which may have peculiar interest. Further details of the investigation will appear later.

THE REDUCTION OF ARSENIC ACID TO ARSENIOUS ACID BY THIOSULPHURIC ACID

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While endeavoring to work out a practicable field method for the estimation of the total arsenic—that is, a method which should include both arsenites and arsenates—in arsenical baths used for dipping cattle, studies were made upon the effect of various reducing agents which are able to absorb iodin in acid solution upon the well-known reversible reaction, $As(OH)_3 + 2I + 2H_2O \rightleftarrows As(OH)_5 + 2HI$. Unless the solution in which this reaction is taking place is freely acidified with a strong mineral acid or heated, the progress of the reaction from right to left is inconveniently slow. It was found that the addition of sodium thiosulphate apparently so greatly aided the reduction that it rapidly went to completion, even in cold and but slightly acid solutions. From this observation it was but one step to discover that the presence of hydriodic acid played no part whatever, the reduction of arsenic acid to arsenious acid being quickly and completely effected by treatment with a mixture of sodium thiosulphate and mineral acid alone.

It has long been known that arsenic, like some other metals, may be quantitatively precipitated as sulphid by sodium thiosulphate in a boiling acid solution. In the present case, however, provided the conditions are right, there is no formation of arsenious sulphid.

The reactions which may follow from the acidification of a solution of sodium thiosulphate are complex and variable, depending upon temperature, dilution, relative proportions of thiosulphate and acid, and possibly upon the order in which the admixture is made. The matter has most recently been discussed by Stiasny and Das,[1] who studied the reactions between such a mixture and potassium bichromate, a problem similar in nature to the one here under consideration.

Preliminary experiments showed that (1) the rapidity with which the reduction of arsenic acid progresses is mainly dependent upon the concentration of hydrogen ions, the organic acids, except oxalic, operating very sluggishly, and (2) the nature of the reactions probably depends to a considerable extent upon whether arsenic or thiosulphuric acid is in excess and is also varied by the order in which the three components, arsenic acid, thiosulphate, and mineral acid, are mixed if the operation of mixing occupies any considerable time.

The present series of experiments was limited to the study of the reactions occurring when a mixture of arsenic acid, or arsenate, with excess of sodium thiosulphate is acidified with an appropriate amount of hydrochloric or sulphuric acid, such being the conditions which must necessarily prevail in any method for the quantitative estimation of arsenic which might be based on the reactions. The solutions employed were

[1] Stiasny, Edmund, and Das, B. M. Reaction between sodium thiosulphate and a mixture of potassium bichromate and sulphuric acid. A contribution to the chemistry of chrome tannage. Jour. Soc. Chem. Indus., v. 31, no. 16, pp. 753-759. 1912.

(1) a tenth-normal (oxidimetric) solution of arsenic acid prepared by oxidizing arsenious acid with nitric acid and expelling excess of the latter, (2) a tenth-normal solution of sodium thiosulphate, (3) a twentieth-normal solution of iodin, free from iodate, and (4) normal hydrochloric acid. The equivalents of the solutions were as follows:

Ten c. c. of the solution of arsenic acid reduced, after Williamson, with hydrochloric acid and potassium iodid and then rendered alkaline with an excess of sodium bicarbonate required 19.74 c. c. of the iodin solution.

Twenty c. c. of the solution of sodium thiosulphate required 39.50 c. c. of the iodin solution. To the solution of sodium tetrathionate thereby resulting there were added 10 grams of dry sodium carbonate and the solution, loosely covered, was heated one hour upon a steam bath. It was then cooled, diluted, acidified to litmus paper with acetic acid, and without delay titrated with iodin solution, of which 39.45 c. c. were required.

In the experiments to be described a measured quantity of arsenic acid was diluted to 25 c. c. and was mixed—whether previously neutralized or not appeared to be immaterial—with 20 c. c. of thiosulphate added from a burette, and then with 10 c. c. of normal hydrochloric acid added from a pipette. When containing moderate amounts of arsenic, the mixtures remained perfectly clear for possibly 15 minutes, disengaging but a slight odor of sulphur dioxid. After a variable time an opalescence would appear, rapidly increasing and becoming yellow and accompanied by a pronounced odor of sulphur dioxid. For quantitative work the action obviously must be stopped before the separation of sulphur and arsenious sulphid becomes perceptible. From the considerable number of experiments only enough will be described to show the nature of the reactions occurring.

EXPERIMENT No. 1.—Ten c. c. of the solution of arsenic acid, 15 c. c. of water, 20 c. c. of the solution of sodium thiosulphate, and 10 c. c. of hydrochloric acid were mixed and allowed to stand for 7¼ minutes. The solution was then titrated with the iodin solution, using starch indicator (titration I), after which sodium bicarbonate was added, avoiding unnecessary excess, and titration with iodin continued (titration II). The end point of titration II was but briefly persistent, owing to the tendency of sodium tetrathionate to be oxidized to sulphate by iodin in alkaline solution. Next, 10 grams of dry sodium carbonate were added and the solution, loosely covered, was heated for 1 hour on a steam bath. Then it was cooled, somewhat diluted, acidified to litmus paper by acetic acid, and immediately titrated again with iodin (titration III). The results obtained were as follows:

Titration I	20.50 c. c. of iodin.
Titration II	19.75 c. c. of iodin.
Titration III	35.25 c. c. of iodin.

EXPERIMENT No. 2.—Experiment No. 1 was duplicated, with the single exception that the mixture was allowed to stand but 2½ minutes before titration I was started. The results were as follows:

Titration I	20.55 c. c. of iodin.
Titration II	19.80 c. c. of iodin.
Titration III	35.55 c. c. of iodin.

Titration I removes the excess of reducing agent without affecting any arsenious oxid present, provided a sufficient quantity of hydriodic acid be also contained in the solution. To insure this condition, it is safer to add a little potassium iodid just before beginning titration I, though in case of experiments Nos. 1 and 2 sufficient was introduced during the titration itself. Titration II measures the arsenious acid formed by the reduction of arsenic acid.

Comparing now the results of titration II with the iodin equivalent of the arsenic-acid solution, it is evident that the reaction is quantitative as respects arsenic. Comparing the sums of titrations I and II (1) 40.25 and (2) 40.35 c. c.) with the iodin equivalent of the thiosulphate solution (39.50 c. c.), it appears that the formation of

sulphurous acid is very slight and that the essential reaction involves the formation of tetrathionic acid, as follows:

$$As(OH)_5+2H_2S_2O_3=As(OH)_3+H_2S_4O_6+2H_2O.$$

A notable formation of any other acids of sulphur would necessarily result in a markedly higher figure for titration I.

Corroborative evidence of the essential transformation of thiosulphuric acid to tetrathionic acid is given by titration III, for Stiasny and Das have shown that an alkali tetrathionate, heated with sodium or potassium carbonate, is nearly quantitatively reconverted to thiosulphate. Titration III shows reformation of a quantity of thiosulphate equivalent to (1) 35.25 c. c. and (2) 35.55 c. c. of iodin solution, compared with an originally introduced quantity of thiosulphate equivalent to 39.50 c. c. of iodin, which amount of thiosulphate, as already noted, after oxidation to tetrathionate, digestion with sodium carbonate, and repeated titration, required 39.45 c. c. of iodin solution.

To further prove the actual reduction of arsenic acid and also that such reduction is brought about by thiosulphuric acid in the absence of hydriodic acid, the theoretically possible action of which is not rigorously excluded by the conditions of experiments Nos. 1 and 2, the following experiment was performed:

EXPERIMENT No. 3.—A mixture of arsenic acid, sodium thiosulphate, and hydrochloric acid, made exactly as described in experiments Nos. 1 and 2, was allowed to stand for five minutes. After the addition of methyl orange, normal caustic soda was run in until only faint acidity remained, as shown by the orange tint of the solution. After the addition of a little sodium acetate and a drop or two of acetic acid to insure a distinctly acid reaction to litmus paper the solution was titrated cold with uranium acetate, using potassium ferrocyanid as indicator. The end point was reached upon the addition of 1 c. c. of uranium-acetate solution. Five c. c. of the arsenic-acid solution was then added and titration continued. The end point was again reached upon the addition of 10 c. c. more of uranium acetate, or a total of 11 c. c. Lastly, 5 c. c. of the arsenic-acid solution, treated in a parallel manner, but without any addition of sodium thiosulphate, required 10.75 c. c. of uranium-acetate solution. The previous conclusions regarding the nature and extent of the action upon arsenic acid were therefore confirmed.

As previously indicated, a small amount of the thiosulphuric acid suffers decomposition into sulphur dioxid and, presumably, sulphur. The sulphur does not become evident under the conditions observed, being partly held in colloidal solution, but for the most part reacting with tetrathionic acid to form pentathionic acid, as shown by Stiasny and Das in their investigations already mentioned. The presence of pentathionic acid was here shown in a similar manner on several of the mixtures while they still remained clear by neutralizing with caustic alkali, using methyl orange as indicator. As the neutral point was reached, a distinct opalescence appeared which was not affected by hydrochloric acid, but which was dissolved after a time by excess of caustic alkali.

The action of thiosulphuric acid upon arsenic acid appears, therefore, at least under the particular conditions studied, to be closely parallel to the action of thiosulphuric acid upon bichromic acid as described by Stiasny and Das.

For obvious reasons it is not likely that the reaction here noted, apparently for the first time, will afford the basis for a desirable volumetric method for use in the laboratory. It may be of value as a convenient means for reducing arsenic acid to arsenious acid preliminary to precipitation by hydrogen sulphid. As a basis for a field test, in default of anything better, it does offer some promise, and experiments in that direction are now under way.

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